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ON THE METAMORPHOSIS OF TWO LEPTOCEPHALI FROM THE MADRAS PLANKTON*

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Introduction

A large number of Leptocephali have been described from different parts of the World, but correlation by actual observation on the metamorphosis as done by Grassi and Calandruccio (1896) has been achieved only in a few species. Lea (1913) and Fish (1927) mention about 16 species of eels of which the larval stages are known, most of them being correlated by myotome and vertebral counts, a character which remains constant throughout the life-history of the eels.

The first record of Indian Leptocephali appears to be that made by Kaup (1856), who gave an account of *Leptocephalus acuticaudatus* and *Leptocephalus dussumieri* collected from Malabar, *Leptocephalus marginatus* from Pondicherry and *Leptocephalus taenia* from India. Later, Southwell and Prashad (1919) described *Leptocephalus milnei* and *Leptocephalus vermicularis* obtained from the brackish waters of the Gangetic Delta. Apparently the latter can only be an advanced elver stage of the former, since the myotome numbers of the two are closely similar. A preliminary study of the eel eggs and larvæ of the Madras Coast was made by Aiyar, Unny and Varkey (1944, Abstract). Recently, Gopinath (1946) has

* Work carried out under a scheme of research financed by the Imperial Council of Agricultural Research, New Delhi.

recorded the occurrence of the Leptocephalus and elver stages of *Congrellus anago* along the Trivandrum Coast.

It may be mentioned here that Cantor (1850) described *Leptocephalus dentex* found in a partially digested condition in the stomach of *Johnius diacanthus* at Pinang. Kaup also recorded *Leptocephalus taenia* from the Maldives. Deraniyagala (1934) described some apodal larvæ collected from Ceylon waters.

This being our state of knowledge of the Indian eels and their larvæ, the present investigation is the first attempt to correlate the Leptocephali occurring along the Madras Coast with the adult eels by allowing them to metamorphose in the Laboratory. So far, two types of eel larvæ have been noted to occur commonly in the Madras Plankton during the months January to April. The occurrence of swarms of the Leptocephali of *Muraenesox cinereus* and *Muraena macrura* in the living condition in the Plankton collections made on the 11th April, 1945 and 14th February, 1945 respectively gave a unique opportunity to study the changes undergone by them during metamorphosis. The young eels thus metamorphosed lived in a healthy condition in the Laboratory tanks for about 2½ and 4 months respectively. Based on the characters of the metamorphosed young eels it is possible to say that one type is *Muraenesox cinereus* of the Family Congridæ, characterised by the presence of canine teeth in the front parts of the jaws and on the vomer, and that the other type is *Muraena macrura* of the Family Muraenidæ distinguished by the origin of the well-developed dorsal fin before the gill opening and the ventral fin immediately behind the anus.

Metamorphosis of the Leptocephalus of *Muraenesox cinereus* (Forskål)

Stage I. †(Fig. 1 and Photographs 1 and 2).

	mm.
Total Length	81
Length of Head	5
Length of Trunk	48
Length from Anus to tip of Tail	28
Length from tip of Snout to origin of Dorsal Fin	20
Maximum Height including Vertical Fins ..	11
Maximum Height excluding Vertical Fins ..	10
Total Myotome number	138
Anal opening below Myotome	78

† The stages described are arbitrary and the measurements and the descriptions given are those of the different stages shown in the photographs.

The Leptocephalus is completely transparent with the body strongly compressed, moderately elongate, tapering towards the head and the tail, particularly towards the latter. This is partly due to the presence of long caudal fin rays which are approximately double the size of the rays of the other vertical fins. Head is elongated with a sharply ending snout. Both the jaws are of equal length and carry teeth which are small, pointed and directed forwards. The cleft of the mouth is straight, horizontal and extends to about the same level as the posterior edge of the eye. The alimentary canal is straight and the anus opens to the outside below the 78th myotome. The pectoral fin is small with faint indications of the rays. The following coloration is quite characteristic. Three to four stellate black chromatophores are present on the middle of the sides of the upper jaw placed at regular intervals. In the heart region similar chromatophores numbering four to five are present. In the region of the entire length of the alimentary canal and at the bases of the anal and caudal fins chromatophores are present with no regularity in their arrangement. The dorsal and the pectoral fins are devoid of any chromatophores. An extensively branched chromatophore is present in the middle region of each myocomma from the 17th myotome onwards. These are regularly arranged in a line just below the level of the vertebral column. In the preceding myocommas, there are only three to four chromatophores, which though not regular in their arrangement are in a line with the others.

Stage II. (Fig. 2 and Photograph 2).

	mm.
Total Length	73
Length of Head	6
Length of Trunk	25
Length from Anus to tip of Tail	42
Length from tip of Snout to origin of Dorsal Fin	11·5
Maximum Height including Vertical Fins ..	8
Maximum Height excluding Vertical Fins ..	6·5
Anal opening below Myotome	50

In this stage certain changes have taken place indicating that the larva has begun to metamorphose. The larva in this stage is very active in its movements and is slightly opaque and cannot be regarded as completely transparent. Of the noteworthy changes, the diminution of the height of the larva is very striking. Consequent on this change the dorsal and the ventral fins have become slightly wider and prominent. There is also a proportionate increase in the width of the larva. Changes affecting the shape of the head have commenced, the snout having become very blunt; the

larval teeth have begun to fall off. The anus has shifted forward considerably and occupies a position below the 50th myotome. The blood in this stage is almost colourless except for a slight red patch in the vicinity of the heart. There is also a general increase in pigmentation. In the anterior part of the snout a group of irregular chromatophores has made its appearance. An increase in the number of chromatophores is noted in this stage throughout the length of the alimentary canal. These chromatophores are more concentrated on the dorsal side of the alimentary canal than on the ventral side where they are comparatively few in number and are widely scattered. Along the bases of each of the rays of the anal fin chromatophores have appeared in a row. In addition to these, a row of eight to ten chromatophores is present in the anterior portion of the anal fin at the region of demarcation of the fin from the body. The borders of the dorsal and anal fins contain a few widely scattered irregularly arranged chromatophores On the body, though there is no increase in the number of chromatophores, those that are present on the myocommas of the larva are slightly larger and more prominent than the others.

Stage III. (Fig. 3 and Photograph 2).

	mm.
Total Length	67
Length of Head	6·5
Length of Trunk	18·5
Length from Anus to tip of Tail	42
Length from tip of Snout to origin of Dorsal Fin	10
Maximum Height including Vertical Fins	7·5
Maximum Height excluding Vertical Fins	5
Anal opening below Myotome	44

Remarkable changes in the shape of the head have taken place in this stage. The height of the body has decreased considerably with a proportionate increase in the width. The blood has assumed the bright red colour and the larva has ceased to be transparent. The larval teeth in this stage have completely dropped out. This and the subsequent stages are edentulous and the larvæ do not feed during metamorphosis. More chromatophores are added in the region of the snout. A few chromatophores are present in the anterior region of the lower jaw and in the region behind the eyes. Pigment cells have begun to appear on the dorsal half of the body with a concentration at the base of the dorsal fin. On the margins of the dorsal and the anal fins small chromatophores are present giving an indication of their coloration in the adult condition.

Stage IV. (Fig. 4 and Photograph 2).

	mm.
Total Length	68
Length of Head	6·5
Length of Trunk	20·5
Length from Anus to tip of Tail	41
Length from tip of Snout to origin of Dorsal Fin	9
Maximum Height including Vertical Fins ..	7
Maximum Height excluding Vertical Fins ..	4·5
Anal opening below Myotome	43

The shape of the head has changed considerably, the blunt snout giving the appearance of an adult eel. The height of the body has decreased further with an increase in width. The body has become completely opaque and white in colour. The larva in this stage is very active and swims about in the aquarium with great rapidity. The anus has shifted still further and is under the 43rd myotome. The pigmentation is more pronounced in this stage. The tip of the snout is dark due to the accumulation of chromatophores. Similarly on the tip of the lower jaw chromatophores have begun to concentrate. Groups of chromatophores are present on the dorsal side of the head and behind the eyes. There is an intensification of the uniformly spread chromatophores in this stage on the dorsal side of the body at the base of the dorsal fin and these chromatophores on the sides are arranged along the myocommas only. A few scattered chromatophores are also present on the myotomes. Numerous chromatophores have formed on the edges of the dorsal and anal fins giving a shaded appearance to the borders of these fins. A few chromatophores are found amidst the caudal fin rays.

Stage V. (Fig. 5 and Photograph 2).

	mm.
Total Length	61
Length of Head	7
Length of Trunk	14
Length from Anus to tip of Tail	40
Length from tip of Snout to origin of Dorsal Fin	7·5
Maximum Height including Vertical Fins ..	6
Maximum Height excluding Vertical Fins ..	3

In this stage the metamorphosis is almost complete and the adult characters predominate except for the colour. An indication of the adult coloration is discernible even in this stage. The movement of the animal in a serpentine manner and darting away at the slightest disturbance is a

very much like the adult eel. The body is muscular and more or less cylindrical in shape. The adult set of teeth has formed but they are very minute in size. The coloration is prominent owing to the new chromatophores

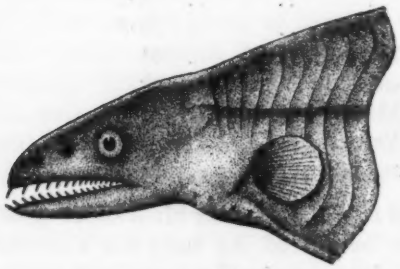

FIG. 1

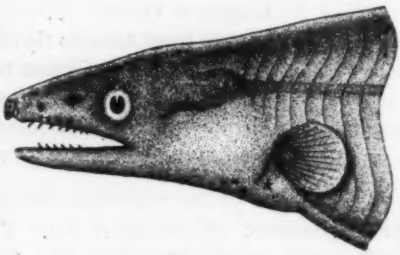

FIG. 2

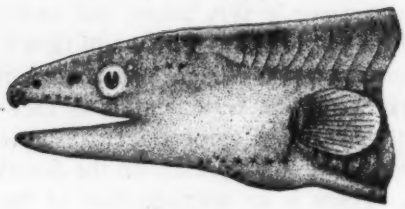

FIG. 3

FIG. 4

FIG. 5

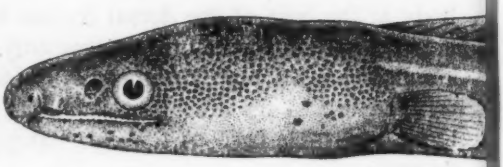

FIG. 6

FIGS. 1–6. Head region of the six stages in the metamorphosis of the Leptocephalus of *Muraenesox cinereus*. × *Ca.* 7.

which have appeared and by the spreading of the chromatophores already present. The coloration is brighter on the dorsal side of the head and the body and also on the margins of the vertical fins and the caudal fin. On the ventral half of the body also pigment cells have appeared particularly along the myocommas. This is more pronounced on the posterior half of the body. The abdomen is free from chromatophores. This pigmentation is very light when compared with that of the dorsal half of the body. A narrow band without any pigment runs throughout the length of the animal along the lateral line. This band gradually disappears in the tail region.

Stage VI. (Fig. 6 and Photograph 2).

	mm.
Total Length	62
Length of Head	8·5
Length of Trunk	12·5
Length from Anus to tip of Tail	41
Length from tip of Snout to origin of Dorsal Fin	8·5
Maximum Height including Vertical Fins ..	4·5
Maximum Height excluding Vertical Fins ..	2·75

This stage could be considered as the juvenile eel. Most of the chromatophores of the dorsal side of the head and the body have enlarged and fused with one another with the result that the young eel is uniformly pigmented when viewed from the top, being brownish black with the ventral portion of the head and the abdomen almost free from pigments. The lateral line is indicated by a thin unpigmented streak. The adult set of teeth is prominent.

The young eels were fed on the flesh of prawn and they grew rapidly. One eel lived in the Laboratory tank for about 2½ months and its rate of growth is as follows:—

1 month old eel	120 mm. in total length.
2 month old eel	160 mm. in total length.
2½ month old eel	180 mm. in total length.

In the last stage the characteristic silvery coloration has begun to appear particularly on the abdomen of the eel.

The important changes that take place in the larva of *Muraenesox cinereus* during metamorphosis may be summarised as follows :—

The loss of the transparency of the larva. The loss of the larval set of teeth at the commencement of metamorphosis. The formation of the adult set of teeth after the completion of metamorphosis. The gradual assumption of the adult coloration by the formation of stellate brownish black chromatophores. The gradual acquisition of the red colour of the blood. The shifting of the anus and the origin of the dorsal fin to an anterior position. The decrease of the body height with the vertical fins becoming more prominent and with a proportionate increase in the width of the animal. The changes affecting the shape of the head.

It is interesting to mention here that these changes resulting in the complete metamorphosis are undergone in the short time of about ten days,

METAMORPHOSIS OF THE LEPTOCEPHALUS OF *Muraena macrura* BLEEKER

Stage I. (Fig. 7 and Photograph 3).

	mm.
Total Length	93
Length of Head	4
Length of Trunk	58
Length from Anus to tip of Tail	31
Length from tip of Snout to origin of Dorsal Fin	50
Maximum Height including Vertical Fins ..	13
Maximum Height excluding Vertical Fins ..	12·5
Total Myotome Number	216
Anal opening below Myotome	120

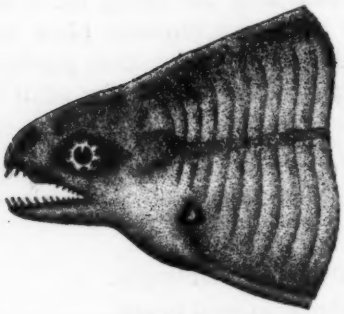

FIG. 7

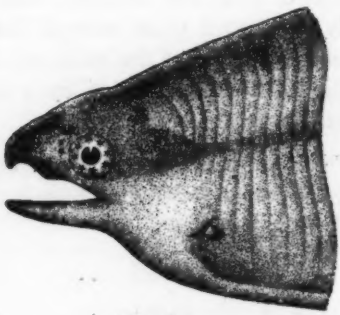

FIG. 8

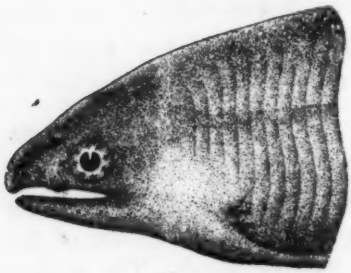

FIG. 9

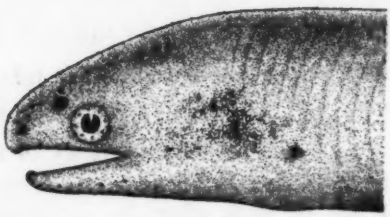

FIG. 10

FIG. 11

FIGS. 7–11. Head region of the five stages in the metamorphosis of the Leptocephalus of *Muraena macrura*. × *Ca.* 7.

The larva of *Muraena macrura* can be easily distinguished from that of *Muraenesox cinerus* by certain characteristic features. The larvæ are completely transparent and are very slow in their movements. It is not quite easy to locate them in water and the only sign to detect them is the prominent colour of the eyes. When disturbed they roll sideways to form a spiral with the head in the centre, which may be another device for protection in addition to transparency. Unlike the other Leptocephali they seldom make an attempt to swim away.

The body is remarkably thin and high and has a leafy appearance. The two extremities are quite blunt and especially so in the caudal region. The head is roughly conical with a blunt snout. The lower jaw is pointed and is of about the same length as the upper one. Both jaws carry strongly pointed teeth which are directed forwards. The cleft of the mouth is straight and extends to about the centre of the eye. The alimentary canal has a straight course and opens to the outside below the 120th myotome. Though the adults are without the pectoral fins they are present in the larval stages very near the gill opening. The coloration is constant in this species particularly in the region of the head. The eyes are coloured golden yellow and round the central circular black region are placed eight, large, stellate, black chromatophores of uniform size. These chromatophores are regularly arranged and are placed equidistant from one another. The golden yellow coloration is also present as patches in the anterior and posterior regions adjacent to the eye. The posterior patch is larger than the anterior one. Black stellate chromatophores are present on the middle dorsal region of the alimentary canal. At the bases of the anal fin, the caudal fin and the posterior third of the dorsal fin similar chromatophores are present arranged in a line. These chromatophores are not conspicuous in the living condition but could be made out only in the preserved condition with the aid of a magnifier.

Stage II. (Fig. 8 and Photograph 3).

	mm.
Total Length	77
Length of Head	5
Length of Trunk	48
Length from Anus to tip of Tail	24
Length from tip of Snout to origin of Dorsal Fin	40
Maximum Height including Vertical Fins ..	11·5
Maximum Height excluding Vertical Fins ..	11
Anal opening below Myotome	120

This stage shows the commencement of the metamorphosis of the larva. The larval teeth are shed completely and the larvæ remain edentulous for

the duration of metamorphosis and apparently without taking any food. The pectoral fins have begun to show signs of atrophy and in this stage they are considerably smaller than those of the preceding stage. The only other marked change is the reduction in the height of the larva. There is no shortening of the alimentary canal in this stage and the anus is under the same myotome as before. But for the appearance of three to four small chromatophores in the region of the snout and a slight opacity, the coloration of the larva remains unchanged.

Stage III. (Fig. 9 and Photograph 3).

	mm.
Total Length	80
Length of Head	5·5
Length of Trunk	47·5
Length from Anus to tip of Tail	27
Length from tip of Snout to origin of Dorsal Fin	40
Maximum Height including Vertical Fins ..	8·5
Maximum Height excluding Vertical Fins ..	8
Anal opening below Myotome	120

Many changes have taken place in this stage of which those affecting the shape of the head are especially noted. The jaws, particularly the upper one, have elongated and become more pointed. The cleft of the mouth has increased in length and extends to about the posterior border of the eye. The height of the body has decreased considerably. There is no anterior shifting of the anus and its position is the same as in the previous stages. The blood is faintly red and the larvæ are more or less opaque. They are more active than before and the slightest disturbance makes them swim rapidly in the aquarium. The rolling habit of the larval stage is no longer observed. Pigmentation remains unchanged except for the appearance of a few more chromatophores in the upper jaw and a few pigment cells in the lower jaw.

Stage IV. (Fig. 10 and Photograph 3).

	mm.
Total Length	78·5
Length of Head	6·5
Length of Trunk	40
Length from Anus to tip of Tail	32
Length from tip of Snout to origin of Dorsal Fin	30
Maximum Height including Vertical Fins ..	6·5
Maximum Height excluding Vertical Fins ..	6
Anal opening below Myotome	103

The height of the body has diminished to a marked extent with a proportionate increase in the width of the animal. The head has almost assumed the shape of the adult eel with the cleft of the mouth extending beyond the posterior limit of the eye. The prominent pigmentation of the eyes has begun to disappear due to the gradual darkening of the peripheral region. The pectoral fins are represented in this stage as mere vestiges near the border of the gill opening. Only from this stage the anus begins to move forward to occupy an anterior position being situated in this stage under myotome 103. The blood is brightly coloured and the vicinity of the heart is bright red. The transparency of the larva is completely lost, the metamorphosing one being perfectly opaque. The larvæ are very active in their movements. Brown chromatophores have appeared uniformly all over the head and the body with a concentration on the head and the vertical fins thus giving an indication of the adult coloration.

Stage V. (Fig. 11 and Photograph 3).

	mm.
Total Length	69
Length of Head	9
Length of Trunk	23
Length form Anus to tip of Tail	37
Length from tip of Snout to origin of Dorsal Fin	8
Maximum Height including Vertical Fins ..	4·5
Maximum Height excluding Vertical Fins ..	3

This is the final stage in the metamorphosis of the larva where the adult characters are assumed in all respects and the typical appearance of the Muraenid eel is reached even in regard to coloration. The head has transformed completely with the cleft of the mouth extending posterior to the eye to about an equal distance as the length of the snout. The larval coloration of the eyes is completely lost and they are dark in colour. The adult set of teeth has appeared. The pectoral fins are completely lost and no trace of them could be seen in this stage. The body is perfectly cylindrical due to the great reduction in the height of the larva. The anus has shifted still anteriorly over a considerable distance. The vertical fins have become very broad and prominent. The young eel is uniformly brownish black due to the presence of numerous closely set stellate chromatophores.

These also feed voraciously on the flesh of prawns. Growth is rapid and the rate in one specimen is as follows;—

1 month old eel	..	.. 120 mm. in total length.
2 month old eel	..	.. 160 mm. in total length.
3 month old eel	..	.. 200 mm. in total length.
4 month old eel	..	.. 240 mm. in total length.

As they grow the young eels become dark brownish black in colour (Photograph 4).

Reviewing the changes undergone during the metamorphosis of this Muraenid larva into the adult eel, we find that these are essentially the same as those of *Muraenesox cinereus* with some characteristic differences. The noteworthy changes are:—

The loss of the transparency of the larva. The loss of the larval set of teeth at the commencement of metamorphosis and the edentulous condition of the larva till the assumption of the adult characters. The gradual atrophy and the complete loss of the pectoral fins. The gradual assumption of the red colour of the blood. The shifting of the origin of the dorsal fin to an anterior position and the elongation of the head. The decrease of the body height to a considerable extent leading to the cylindrical shape of the eel. Unlike *Muraenesox cinereus*, the adult pigmentation is not observed in the first three stages but when the larva reaches the fourth stage the adult pattern is rapidly formed. The same is observed with regard to the anterior shifting of the anus which remains in the same position in the first three stages. The anus moves forward over a considerable distance during the last two stages. The vertical fins become wide and prominent only in the last stage of metamorphosis. The time taken for complete metamorphosis is about ten days which is about the same as that taken by *Muraenesox cinereus*.

General Remarks

It is well known that the European eel, *Anguilla vulgaris* and the Americal eel, *Anguilla rostrata*, migrate to the common breeding place in the Western Atlantic and that their larvæ, *Leptocephalus brevirostris* and *Leptocephalus grassi*, have three and one years of pelagic larval life respectively before they reach their respective Coasts. Very little is known about the breeding places of the Indian eels. Deraniyagala (1929) has collected the elvers of *Anguilla bicolor* and *Anguilla elphinstonei* from the Pearl Banks and suggests (1934) the possibility of Ceylon Anguillidæ reproducing throughout the year in the Coastal waters. According to Schmidt (Deraniyagala, 1934) the sea west of Sumatra is the breeding ground of eels and from here he has collected the tiny larvæ of *Anguilla bicolor* and *Anguilla elphinstonei*. He considers that the larvæ approach the Coast only at the end of their

pelagic life. Rahimullah, Mahmood and Kabir (1944) are of opinion that *Anguilla bengalensis* breed in freshwater leaving its catadromous breeding habits.

From the Madras Plankton only the final pelagic larval stages of the two Leptocephali studied have so far been collected though regular collections have been made during the past ten years.* If the eels reproduce throughout the year in the Coastal waters as suggested by Deraniyagala, it should be possible to get at least a few of the earlier stages of the larvæ especially when they occur in enormous numbers. This seems to show that the eels do not breed near the Madras Coast and that probably their breeding place is the open sea.

Grassi and Calandruccio (1896) have shown that *Leptocephalus brevirostris* takes about a month for the transformation into the elvers in the aquarium at Naples. The metamorphosis is much quicker in the two forms studied at Madras as they take only ten days to complete the metamorphosis. This is to be expected owing to the higher temperature conditions in which these Leptocephali grew and metamorphosed.

ACKNOWLEDGEMENT

My thanks are due to Dr. N. Kesava Panikkar, who was formerly the Director of the University Zoological Research Laboratory, Madras, for valuable help and suggestions.

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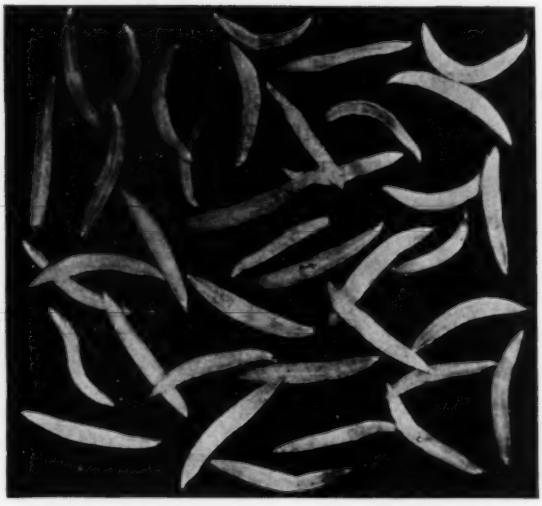
Explanation of Photographs

1. A small portion of the swarm of the Leptocephalus of *Muraenesox cinereus* collected on the 11th April, 1945. × *Ca.* 3/10.
2. The six stages in the metamorphosis of the Leptocephalus of *Muraenesox cinereus*. About nat. size.
3. The five stages in the metamorphosis of the Leptocephalus of *Muraena macrura*. About nat. size.
4. Four month old *Muraena macrura*. × *Ca.* 3/8.



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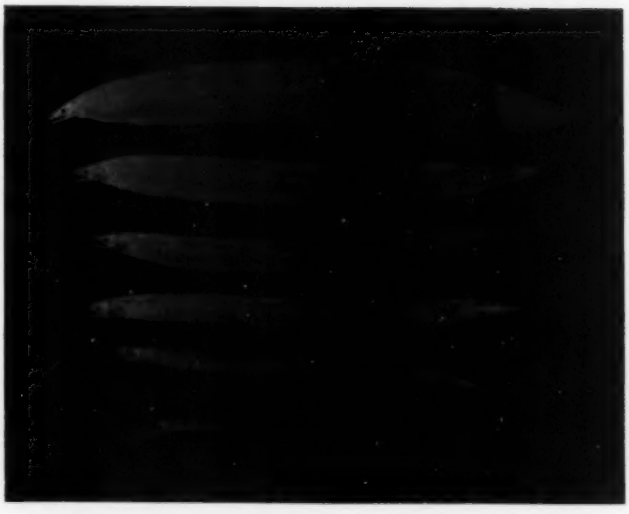

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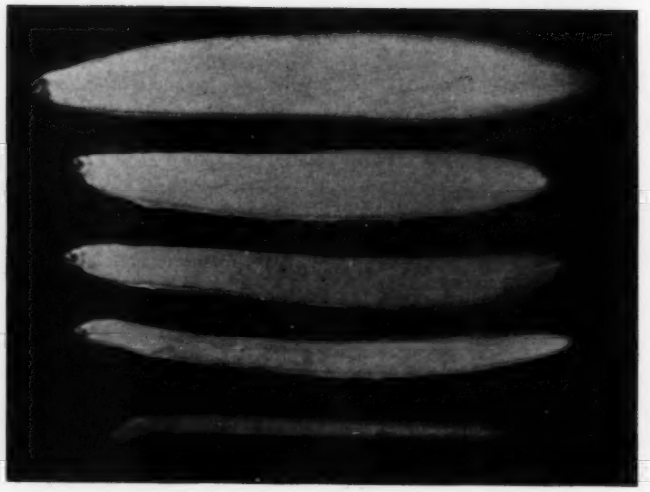

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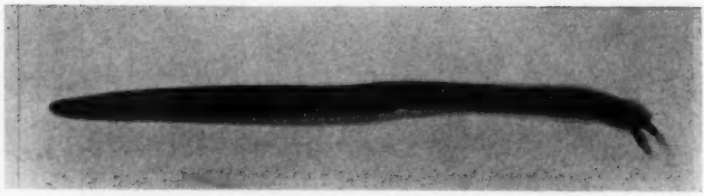

4

STUDIES IN THE GENUS COLLETOTRICHUM—III

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In earlier communications (Ramakrishnan, 1941)[1,2] the parasitism of *Colletotrichum indicum* and the occurrence of saltations in *C. capsici* Syd. have been dealt with. During the course of further investigations it was observed that a close resemblance existed between these two species and some other isolates belonging to this genus. The results of these investigations embodying the studies of certain aspects of the physiology of *C. indicum* and the comparison of the isolates from *Capsicuum annum* (*C. capsici*), *Curcuma longa* Syd. (*C. curcumæ* Syd.), *Aristolochia bracteata* Retz. and *Cicer arietinum* (*C. capsici*) are recorded in this paper.

Materials and Methods

The isolate of *C. indicum* Dast. obtained from specimens sent by Prof. Dastur in 1938 was used. *C. capsici* was isolated from diseased specimens of *Capsicum annuum* from the Agricultural Research Station, Taliparamba (Malabar). Sundararaman (1930) and Thomas (1941) have recorded the occurrence of *Colletotrichum* on *Aristolochia bracteata* in Coimbatore. The fungus was isolated from fresh leaf-spots on this host. Fresh specimens of diseased leaves of *Curcuma longa* were obtained from Bhavani (Coimbatore District) through the courtesy of Sri. A. Rathnavelu, the Agricultural Demonstrator, Bhavani, and the fungus *C. curcumæ* was obtained from these. *Cicer arietinum* was affected by a blight at Pollachi (Coimbatore). This was found to be due to *Colletotrichum* and this isolate was also included in the comparative studies. Sundararaman (1926) has recorded *Colletotrichum* (*Vermicularia*) on *Cicer arietinum.*

The cultures of the isolates were initiated from single spores and maintained on either oat agar or french bean agar. Petri-dish cultures were grown inside an incubator at 32° C. unless otherwise stated. The dry weights of the fungus growths and the reaction of the fungus to different sources of nitrogen and carbon were determined following the method described by Ramakrishnan (1941.)[3] For all inoculation experiments on cotton *G. herbaceum* strain H_1 was employed.

A. Physiology of C. indicum.

Temperature relations.—The isolate grows well on agar media at the laboratory temperature (27° to 30° C.). The relative growth and sporulation at other temperatures also were studied. The fungus was inoculated into Petri-dishes containing oat agar and french bean agar and the dishes were transferred to controlled temperature chambers where the temperatures were maintained at 5°, 10°, 15°, 20°, 32°, 37° and 44° C. respectively. Closer intervals could not be utilised. The results were as follows:—

TABLE I

Temperature	*Oat agar*		*French bean agar*	
	Diameter in mm. in 7 days	Nature of growth	Diameter in mm. in 7 days	Nature of growth
5° C.		No growth.		
10° C.	Slight development		Not measurable.	
15° C.	31·3	Dark growth, black stromata in the centre, very few acervuli.	26·0	Dark, thin growth few acervuli.
20° C.	63·5	Olive-black growth, grey aerial mycelium, fair production of acervuli.	28·5	Black centre, lighter outside, numerous acervuli.
32° C.	80·0	Aerial mycelium pale olive grey, numerous buff pink acervuli.	73·7	White and grey aerial growth, numerous black stromata and pink acervuli.
37° C.	62·3	Acervuli fewer.	54·25	Less of aerial growth and fewer acervuli.
44° C.		No growth.		

The best growth occurs in the neighbourhood of 32° C. among the temperatures under trial. Sporulation is evident between 15° and 37° C. and the maximum acervular development is at about 32° C. There was no development at 5° C. but the fungus remained quiescent. When the dish was transferred from 5° C. to the laboratory temperature after one week, the fungus began to grow again and covered the dish in 8 days. The dishes kept at 44° C. did not exhibit any growth and even after removal to the laboratory temperature a week later there was no revival of the fungus. Continuous exposure to 44° C. for a week had evidently killed the fungus. Ling and Yang (1941) have found that the Chinese isolate of *C. indicum* grew best at 28° C. This temperature, however, was not included in the experiments conducted here and therefore it cannot be said that the optimum temperature for the local isolate is different. But the same authors have

also found that even in the Chinese isolate the highest germination of spores and the maximum length of germ tubes are at 32° C.

The dry weights of fungus mats grown in liquid cultures at different temperatures were also recorded. The results are given below.

TABLE II

Temperature	Dry weight in milligrammes of fungus mat (17 days' old)
10° C.	43·9
15° C.	243·0
20° C.	245·6
26°5 C.	265·3
32° C.	315·2
37° C.	155·2

These results show that liquid cultures follow a similar trend as the dish cultures.

Temperature is known to influence the spore size in some fungi (Johann, 1913, Ramakrishnan, 1941, 3). Measurements of spores from the cultures kept at different temperatures were taken and the mean length and frequency distribution are given below.

TABLE III

Class in μ	Frequency distribution at						
	15° C.		20° C.		32° C.		37° C.
	Oat agar.	F. bean agar.	Oat agar.	F. bean agar.	Oat agar.	F. bean agar.	Oat agar.
16—20	3	3	9	3	16	11	19
21—25	95	102	111	103	111	95	99
26—30	96	86	77	88	68	91	81
31—35	6	9	3	4	5	3	1
Mean length in μ	26·1	26·0	25·5	25·6	25·2	25·6	25·2

The spore length has been remarkably constant at all temperatures in this isolate.

The optimum temperature for infection of cotton was determined. Cotton seeds soaked for one hour in a spore suspension in distilled water were sown in pots containing sterilized soil. Twenty seeds were sown in each treatment. The pots were kept in chambers with air temperatures at 15°, 20°, 30° and 35° C. respectively. Control pots containing sterilised soil sown with uninfected healthy seeds were also kept. The pots were kept under observation for one week and the following results were obtained.

TABLE IV

Temperature	Inoculated		Control	
	No. germinated	No. of seedlings infected	No. germinated	No. of seedlings dead
15° C.	5	1	6	—
20° C.	11	8	12	—
30° C.	14	14	16	—
35° C.	13	4	16	—

Among the temperatures included in the experiment mortality is high at 30° C.

Carbon and nitrogen sources on growth and sporulation.—Different sources of carbon and nitrogen are known to influence the growth and sporulation of *Colletotrichum* in different ways. To ascertain whether this isolate also behaves in a similar manner it was grown on media having a basic composition (Ramakrishnan, 1941, 3) to which equivalent weights of different carbohydrates or nitrogenous substances were added.

Carbohydrates.—The fungus was grown on solid and liquid media. The average diameter of the growth after 7 days and the average dry weight of the fungus mat in liquid media after 17 days were determined.

TABLE V

Statement showing the diameter of growth or dry weight in different carbon sources

Carbon source	Agar media		Liquid media		
	Diameter in mm. 7 days	Remarks	pH at start	pH after 17 days	Weight of dry mat in mg.
Sucrose ..	68·3	Numerous black and light vinaceous fawn acervuli with spore masses.	4·4	6·8	266·7
Glucose ..	68·5	Black sclerotioid bodies and big light vinaceous fawn spore-bearing acervuli.	4·3	7·3	223·3
Maltose ..	80·3	Black sclerotioid bodies, acervuli less than in sucrose.	4·4	7·3	270·9
Lactose ..	71·0	Thin growth, black sclerotioid bodies formed, few acervuli.	4·4	6·0	107·6
Starch (soluble)	74·5	Thin white growth with a number of drab masses. Acervuli more than in lactose.	4·6	7·2	188·4

Maltose and sucrose induce good growth but sporulation is best in sucrose.

TABLE VI

Statement showing the growth of the fungus and spore length on different sources of nitrogen

Source of Nitrogen	Diameter of growth in 8 days mm.	Spore length in microns		REMARKS
		Range	Mean	
Peptone ..	60·8	20–36	27·7	Smoke grey growth, numerous pale vinaceous acervuli all over the growth.
Asparagin ..	43·5	20–32	25·8	Pale smoke grey aerial growth margin regular, numerous acervuli and black stromatoid bodies all over the growth.
Potassium nitrate ..	45·5	20–36	27·4	Pale smoke grey aerial growth, numerous black stromatoid bodies acervuli scattered in growth.
Ammonium sulphate ..	19·7	42–28	24·2	Thick growth, margin crenate, and ridged, pale olive grey aerial growth, very few acervuli.
Urea ..	32·5	16–32	23·3	Mealy, pale greyish vinaceous growth, acervuli more than in ammonium sulphate, spore vacuolated.
Potassium nitrate ..	—	No growth.		

Peptone serves as a good source of nitrogen. The growth is slow and sporulation less when ammonium sulphate or urea are used.

Staling.—The uniform rate of growth of the fungus for 10 to 12 days on agar media, does not suggest any accumulation of staling products in the early stages of its growth. But in liquid cultures maintained for over three weeks there is evidence of the development of staling products, as no further increases in weight of fungus were obtained. In order to clear this point the fungus was grown on filtrates from cultures 25 days old. The filtrate was mixed with fresh Richards solution in the proportion of 1 : 1 and autoclaved before use. The control consisted of Richards solution mixed with an equivalent volume of distilled water before autoclaving. The two sets of media were inoculated from the same culture with equal quantities of inoculum. After fifteen days' growth the fungus mat was removed and the dry weight determined. The weights were as follows:—

TABBLE VII

Medium used	Average dry weight of fungus mat in mgm.
Filtrate from culture of *C. indicum* + Richards solution ..	50·8
Richards solution + Distilled water ..	169·7

From the above it is evident that staling products accumulate in cultures over three weeks old and these inhibit the growth of the organism. The presence of these substances was further demonstrated by allowing fresh seedlings of cotton (H_1 strain) to stand with their roots and hypocotyl immersed in the filtrate (filtered through coarse filter-paper) of cultures 25 days old. The controls were kept with the roots immersed in Richard's solution adjusted to the same pH as the filtrate. In 12 hours the seedlings kept in the filtrate wilted while the controls were quite turgid (Plate III, Fig. 4). Ling and Yang (1944) state that they were not able to demonstrate the production of toxic substances. This may be due to the fact that the toxic staling products had not developed in the filtrate from 10-day-old cultures used by them. Under Coimbatore conditions it was observed that the formation of staling products or their accumulation in sufficient quantity becomes evident only in old cultures. Further these authors have been studying an isolate of the fungus prevalent in China. It is quite probable that the Chinese strain and the Indian strain do not behave alike. This view is supported by the observation that the Chinese strain infects two varieties of *G. hirsutum, viz.,* Trice and Delfos—while all the isolates studied in Coimbatore including the strain, kindly supplied by Dastur from Nagpur have not been found to be pathogenic on *G. hirsutum* but only on *G. herbaceum* and *G. arboreum*. This fungus has been under observation in South India for over twenty years and all through this period there has been no record of its occurrence on any strain of *G. hirsutum* though Combodia cotton (*G. hirsutum*) is cultivated over a large area in Coimbatore. Thus neither in nature nor by artificial infection was the fungus found to infect *G. hirsutum.* Dastur (1934) who described the fungus from Nagpur has recorded it only on *G. arboreum.* Consequently it is presumed that the Chinese strain behaves differently from the Indian strain of the fungus in some of its physiological reactions.

Saltation.—A number of saltants were developed by this isolate on Richards agar and oat agar in the form of sectors or islands (Plate III, Fig. 3). The saltants exhibited differences in the colour and texture of the growths and in the intensity of sporulation. Non-spore-forming saltants were also formed.

B. Comparative study of C. indicum *with* C. capsici, C. curcumæ *and isolates from gram* (Cicer arietinum) and Aristolichia bracteata.

A comparison of the external morphology of the different isolates under study revealed a very close resemblance to one another. The appearance of the acervuli on the respective hosts was similar. Very often they exhibited

formation in concentric rings. Normally they are black with a well developed stroma which projects outside the host tissue. On the stroma are developed long dark septate setæ mixed with hyaline one-celled conidiophores. Falcate (crescent-shaped) unicellular, hyaline conidia are formed on these conidiophores. When large numbers of spores are formed the spore mass on the acervulus assumes a deep to light pink colour.

The size of the acervulus exhibits a wide variation in the same host, the range of variation being from 45 to 295 μ. The range of variation exhibited by setæ of any one isolate is very great. The size of the setæ in agar cultures also varies within wide limits.

The conidia of all the isolates of the same age were of the same shape. Measurements were taken of 200 conidia of each. The range of variations and the average measurements agree very closely. The following table represents the measurements of the spores of these isolates as compared to the original measurements obtained by different authors.

TABLE VIII

Species or isolate	Size of spore given by original authors		Size of spores found on host tissue Author's measurements	
	Length μ	Breadth μ	Length μ	Breadth μ
C. indicum ..	15–25 (Dastur)	1·8–4·3	24·60 (18–31)	3·1
C. capsici ..	17–28 (Butler)	3–4	25·3 (19–31)	3·2
C. curcumae ..	18–29 (Sundararaman)	3–5	25·4 (17–31)	3·1
C. on *Aristolochia* ..	..	..	24·4 (20–30)	3·2
C. on gram. ..	.. 21–34 (Sundararaman)	.. 3–6	24·5 (22–28)	3·1

(Figures within brackets represent the range of measurements.)

From the above table it can be seen that there is no difference in the spore size between the isolates. On the other hand, there is very close agreement.

On agar media the first generation of the isolates exhibits a medium proportion of pale grey to pale olive grey aerial mycelium and numerous acervuli with pink spore masses. When the same isolate is carried through a number of generations the aerial mycelium diminishes in quantity. Slight differences are noticed between the isolates in the colour developed during the later generations but these fall within the normal variability of the same isolate or may be due to the formation of saltants,

In order to determine the host range of these isolates inoculation experiments were conducted on *G. herbaceum, Capsicum annuum, Cicer arietinum* and *Aristolochia bracteata.* Fifteen inoculations were made in each case on the respective plants and the results recorded after seven days are noted below.

TABLE IX

Statement showing the number of positive infections at the end of seven days

Isolate	Cotton seedlings	*Capsicum* fruits	*Aristolochia* leaves	Gram seedlings
C. indicum ..	15	4	13	15
C. capsici. ..	—	11	14	15
C. curcumae ..	—	10	12	13
C. from *Aristolochia* ..	9	6	14	14
C. from gram ..	8	8	10	14

The controls remained healthy in all cases. The isolates from *Capsicum* and *Curcuma longa* do not affect cotton. All the isolates have infected varying numbers of the other hosts.

Sansome (1938) has described how Reddick was able to improve the parasitism of *Phytophthora infestans.* He found " that the variety of potato President is resistant to *P. infestans.* But after two passages through President by artificial infection the degree of virulence of *P. infestans* is increased so that the lesions formed on President are as large as those formed on a susceptible variety, Green mountain. This higher virulence is kept up even after twenty passages through the susceptible variety." A modified method was adopted to improve the virulence of the isolates of *Colletotrichum.* They were grown on sterilised cotton seeds (strain H_1) of *G. herbaceum* and after five passages through cotton seed, the cultures were used to inoculate cotton seedlings. The results were very interesting.

TABLE X

Statement showing the incidence of infection of cotton seedlings

Isolate	No. of seedlings inoculated	Total number infected on							
		3rd day	4th day	5th day	6th day	7th day	8th day	9th day	10th day
Cotton ..	15	4	15	—	—	—	—	—	—
Capsicum ..	15	—	7	11	13	14	15	—	—
Aristolochia ..	15	—	1	4	6	10	15	—	—
Curcuma ..	15	—	—	—	5	6	6	8	8
Cicer ..	15	—	7	10	14	15	—	—	—
Control ..	15	all healthy.							

The results indicate that all the isolates can be gradually 'educated' to become pathogenic on cotton seedlings which were not being infected originally, by growing the organisms on sterilised cotton seeds for a number of generations. All of them do not become equally virulent and there is a difference in the speed of infection (Plate III, Fig. 7).

Another experiment was conducted in which the cotton isolate was grown on sterilised cotton seed or *Capsicum* fruits for seven generations and then used for virulence tests on cotton seedlings. The following results were obtained.

TABLE XI

Statement showing the virulence of the cotton isolate after passage through cotton or Capsicum

Medium	No. of seedlings inoculated	No. of seedlings infected on									
		3rd day	4th day	5th day	6th day	7th day	8th day	9th day	10th day	11th day	12th day
Cotton seed ..	20	12	20	—	—	—	—	—	—	—	—
Capsicum fruits	20	—	—	—	1	2	4	8	12	15	20
Control ..	20	all healthy									

The results show that the infective capacity of the cotton isolate becomes attenuated when grown on *Capsicum* fruits for a number of generations (Plate III, Fig. 8). When grown on agar media, however, the virulence is maintained for a much longer period.

DISCUSSION

It is evident from the studies described above that the taxonomy of the isolates of *Colletotrichum* under study at present classified as three or more different species, is in need of revision. It is seen that these isolates produce saltants very readily on agar media and such changes are bound to take place in nature also. The factors that have guided the erection of these species shall be reviewed and their validity examined.

The chief characteristics that are taken into consideration in defining species are the morphological characters, the dimension of the reproductive bodies, the acervuli and conidia and the pathogenicity of the isolate. These shall be examined one after another to assess the amount of reliability that can be placed on them.

The morphological characters of the isolates under study resemble one another very closely. If they were not properly labelled it will be difficult to distinguish one isolate from another. Ling and Lin (1944) state that "in comparison with a number of species of *Colletotrichum* such as *C. circinans*

C. indicum, *C. truncatum* and *Glomerella glycines*, *C. capsici* differs from them in no essential way."

The dimensions of the acervulus fluctuate very much in the same isolate and consequently its size is not of much taxonomic value. Butler (1918) has recorded the size of the acervulus of *C. capsici* as 75–120 μ. The acervuli of the same species on the fruits of *Capsicum* collected locally have exhibited a fluctuation of 63 to 295 μ and on agar media the maximum reached was 315 μ. Ling and Lin (1944) state that the size of the acervulus of *C. capsici* on one host varied from 74–187 μ while on another host the variation was from 97–288 μ. A structure which exhibits such wide variation cannot be relied upon for specific differentiation.

The setæ formed on the acervuli have been known to be definitely influenced by the environment and substratum to a large extent. Sometimes their formation itself is suppressed. Ikata (1937) and Ramakrishnan (1941) have indicated that the setæ cannot be considered to be of any consequence for the purpose of specific differentiation. The shape and size of the conidium form important taxonomic characters. In the genus *Colletotrichum* the shape of the spore is useful in distinguishing certain species from others. The spores are either oblong, spindle-shaped, or falcate with tapering or blunt ends in different species, being more or less constant in the same species. The size is however influenced by the substrate and varies within limits. Yet its significance in specific differentiation cannot be ignored. Judged by these standards it is seen that all the isolates under study have similar mean dimensions of conidia and cannot be distinguished from each other either by the shape or size of the conidium.

An undue emphasis has been laid on the pathogenicity of the isolates of this genus in differentiating species. *C. capsici* was first recorded on *Capsicum*. Butler and Bisby (1931) have given a long list of plants serving as hosts for this species. They are: *Capsicum* spp., *Solanum nigrum*, *S. xanthocarpum*, *Datura fastuosa*, *Hibiscus esculuntus*, *Canvalia ensiformis*, fruit of *Vigna catjang*, *Dolichos lab lab*, *Solanum melongena*, *Citrus* sp. and *Carica papaya*. Ramakrishnan (1941) has observed the fungus on *Carthamus tinctorius*. Ling and Lin (1944) have noticed the fungus on fruits of *Lycopersicum esculentum* causing a fruit rot in China. A wide host range is thus established for this species. *C. curcumæ* was described as causing leaf-spot of *Curcuma longa*, to which host it owes its specific name. Sundararaman (1925, 1926) carried out a number of cross-inoculations with this isolate and considered that the fungi on *Capsicum* and *Curcuma longa* belong to the same species.

Sundararaman (1922) has however erected a new species *C. zingiberi* (*Vermicularia zingiberi*) causing leaf-spot of *Zingiber officinale*. His decision was arrived at owing to (*a*) "the difference in the measurements of sporodochia between the *Colletotrichum* (*Vermicularia*) on ginger, turmeric, and chillies; (*b*) the character of the chalmydospores; and (*c*) the negative results in the cross-inoculations on chillies and turmeric." In the paper describing this species the measurements recorded of the acervuli (sporodochia) are 50 to 140 μ for *C. zingiberi* and 35 to 160 μ for *C. curcumæ*. The former comes within the range of the latter and does not exhibit any difference. Appressoria (chlamydospores) are formed in all the isolates under study in the paper and no difference in their formation could be made out. It is questionable whether much importance can be attached to negative results of inoculation. In the absence of a thorough knowledge of the optimum environmental conditions necessary to produce successful infections there is every likelihood of failures of infection. The spore measurements were however found to agree with those of *C. capsici*.

Dastur (1934) has erected a provisional new species of *C. indicum* causing seedling blight of cotton. The only difference he found in this isolate when compared with *C. capsici* was in its pathogenicity. He found that the isolate from cotton did not infect *Capsicum* nor *C. capsici* cotton seedlings. But the infection experiments carried out at Coimbatore with the two fungi have shown that both the isolates can parasitize the two hosts.

It can be seen from the above that the occurrence of the fungi on different hosts and the variation in the pathogenicity of the isolates had prompted the creation of new species of *Colletotrichum*. Species of this genus are not obligate parasites but facultative saprophytes capable of leading a saprophytic existence in nature. Specific differentiation on differences of pathogenicity alone is not a reliable guide with such organisms. The substratum on which the fungus grows for a protracted period has been shown to influence the infective capacity of the isolates of this genus. Therefore the creation of new species on the variation of the pathogenic capabilities alone of the organisms cannot be approved. More reliance has to be placed on stable characters.

It is therefore concluded that all the isolates studied above should be included in one species. According to the rules of botanical nomenclature the name *C. capsici* has to be adopted being the earliest. *C. curcumæ* and *C. indicum* have to be merged into this species. The reasons for creating the species of *C. zingiberi* (Sundararaman, 1922) are not tenable and this fungus has also to be brought under *C. capsici* which it resembles very much.

The author himself has stated that "there is a good deal of similarity among the ginger, chillies, and turmeric *Vermicularias* in point of spore measurements." These must be considered only as strains or races of *C. capsici*. This species has a wide host range, and it produces saltants freely and the different races met with in nature might have arisen in a similar manner. Being associated with a particular host for some period the infective capacity of the race on the particular host becomes pronounced. This accounts for the variability in the pathogenicity of the races.

I am grateful to K. M. Thomas, Esq., Government Mycologist, for all the help rendered in carrying out this investigation.

Summary

Studies on the physiology of *C. indicum* Dast. were made. The fungus grows best in the neighbourhood of 32° C. The size of the conidium is not affected by temperature. Maltose and sucrose form the best sources of carbohydrates among those tried. Peptone serves as a good source of nitrogen. Staling products are formed in cultures over three weeks old. Filtrates of old cultures inhibit the growth of the fungus. Seedlings of cotton kept in these filtrates wilt in twelve hours.

A comparative study of *C. indicum, C. capsici, C. curcumæ* and isolates from gram (*Cicer arietinum*) and *Aristolochia bracteata* was made. It was found that by growing the isolate on the tissues of a particular host for a number of generations its pathogenicity on that host is improved. Thus the various isolates under study were able to infect cotton seedlings when they were grown on sterilised cotton seeds for five generations. The taxonomic position of these isolates is discussed and it is concluded that all of them belong to one species, *C. capsici* Syd.

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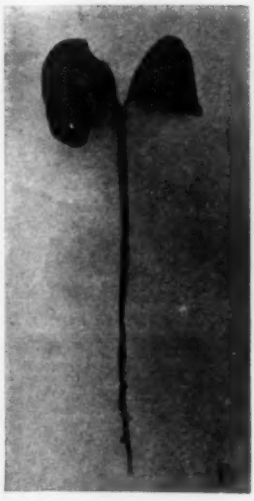

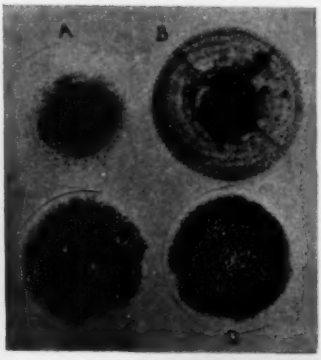
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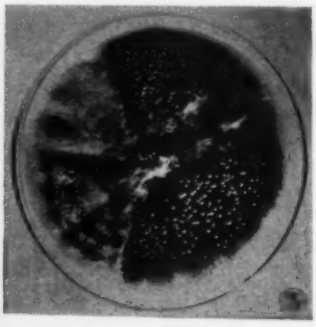

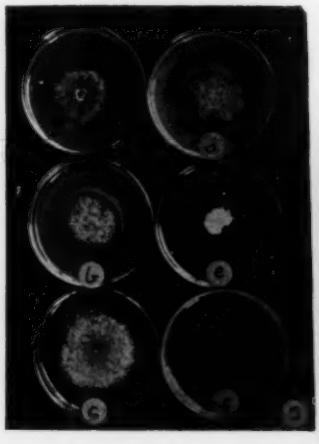

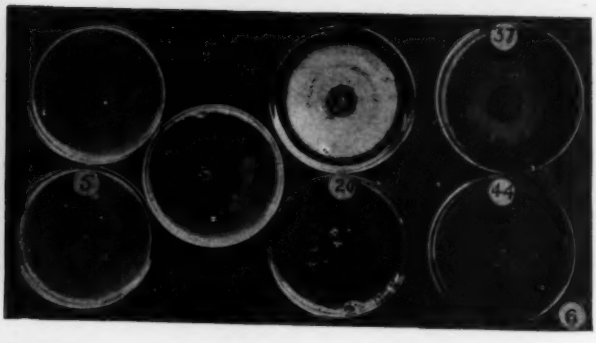
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Explanation of Plate

1. Cotton seedling affected by *Colletotrichum*.
2. Growth of *Colletotrichum* (Cotton strain) on
   (*a*) Lactose. (*b*) Maltose.
   (*c*) Glucose. (*d*) Sucrose.
3. Saltants of *Colletotrichum* (cotton) on Richards agar.
4. Effect of filtrate on cotton seedlings.
   (*a*) Filtrate. (*b*) Richards solution (control).
5. Nitrogen sources and growth of *Colletotrichum* (cotton).
   (*a*) Urea. (*b*) Asparagin. (*c*) Peptone. (*d*) Potassium nitrate.
   (*e*) Ammonium sulphate. (*f*) Potassium nitrite.
6. Effect of temperature on growth.

10° C.	32	37
15° C.		
5° C.	20	44° C.

7. Cotton seedlings infected by (*a*) *C. capsici* (*Capsicum* strain); (*b*) *C. capsici* (*Aristolochia* strain) grown on cotton seeds.
8. Cotton seedlings infected by *Colletotrichum* (cotton strain): (*a*) after 7 passages through *Capsicum*; (*b*) grown on cotton seeds.

ADDITIONS TO FUNGI OF MADRAS—I

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Received October 10, 1946
(Communicated by Dr. B. V. Nath, C.I.E., D.Sc., F.R.I.C., F.A.Sc.)

During a foray undertaken in March 1946, collections of fungi were made in the jungles round about Coonoor and Ootacamund in the Nilgiris district of Madras province. Among the collections were several new records for the locality and some which are new to science. Three of these fungi are described below:

(1) *Xenostele neolitseæ* sp. nov.

Neolitsea zeylanica Merr. is a medium-sized tree common in the upper slopes of the Nilgiris in the neighbourhood of Ootacamund, Kotagiri and Naduvattam. At the time of the visit this plant was affected by a severe epiphytotic of rust in all these places. Numerous brown rounded woody galls were seen on the leaves and sometimes on the petioles and stem (Plate, Fig. 1). The galls on the leaves are more conspicuous on the lower surface, there being few or none on the upper. They are isolated or clustered together and their diameter varies from 2 to 5 mm. But on the branches the hypertrophied portions are 2·5 cm. or more in thickness as against the normal thickness of half a centimeter of the healthy part. Wherever a gall develops on the lower surface of the leaf a corresponding depression is visible on the upper surface.

On the surface of the galls a number of rounded warts or tubercles are seen. The wart is ruptured and a whitish conical structure projects from the interior. In others a depression or cup-like cavity is present from which reddish brown mass of teliospores is visible. Two to thirty-two cups or more can be seen on old galls depending on their size.

The gall is very hard and woody. In cross-section the tissue of the gall is found to be made up of a high proportion of thick-walled scalariform cells mixed with parenchymatous cells packed with starch grains. Sunk in the galls are the cups of the teliosori. In the early stages a conical whitish to cream-coloured solid structure projects out of each sorus, bursting through the outer surface. When the galls are young a number of these whitish structures are seen protruding out. These can be easily picked out by needles and they come off as "stoppers". The conical structure represents the

peridium. When a section is examined the peridium appears as a solid mass made up of several layers of hyaline, thick-walled, sterile cells, rectangular to polygonal in shape and 40–80 × 17–19 μ in size, closely united together and with a finely warty surface. In nature the peridial caps fall off after a time and expose the telia (Plate, Fig. B).

Telia are cup-shaped, 300–400 μ deep and 300–410 μ wide. A pseudo-parenchymatous mass of fungal tissue develops from the bottom of the cup. The upper layers of this tissue are made up of elongated cells closely packed together (Plate, Fig. C). The teliospores originate from these cells. The teliospores are stalked with long hyaline pedicels up to 200 μ in length. The pedicels easily break off leaving short remnants still attached to the spore. Each telium produces several spores borne in succession, the older ones being pushed up as new ones are formed. Thus an apparent resemblance to several layers of spores is produced. The remnants of the basal parts of the pedicels of old spores can be seen between the younger spores. Each teliospore is two-celled, elliptical or spindle-shaped, with rounded ends, dark reddish-brown and smooth-walled. The shape of the teliospore resembles a structure made of two cones united by their bases and having blunt apices. The spores measure 47 × 26 μ (40–56 × 24–29 μ). The upper cell varies in length from 20–24 μ and the lower from 20–32 μ. The cavity of the cells is bell-shaped. One germ pore is present in each cell—at the apex of the top cell and near the point of attachment of the stalk in the bottom cell. There is a slight constriction at the junction of the two cells, which in some cases becomes very pronounced. The two cells may separate from each other or be united only in the centre.

The telial stage alone has been observed in this rust. From its morphology it is manifest that it belongs to the genus *Xenostele* Syd. Two species of this genus have been recorded—*X. echinacea* (Berk) Syd. on *Actinodaphne molochina* in Ceylon and *X. Litseæ* (Pat.) Syd. on *Litsea glauca*—in Japan. The galls formed by *X. echinacea* are developed only on leaves and the telial cups are 200–250 μ in diameter. Further the stalks of the spore are said to be golden-brown and twisted into bundles. The rust on *Neolitsea* produces galls on stems and leaves and the cups are bigger being 300–410 μ wide. The stalks are hyaline and not twisted into bundles. *X. Litseæ* has been reported only on the leaves of *Litsea glauca* and the spores have a rough surface as described by Sydow (1920). The spores of the rust on *Neolitsea* are quite smooth and no warts have been seen even when they are examined with an oil-immersion objective. Since the host is different and there are differences in the size of the sorus, the nature of the pedicel and the wall of

the spore this rust is considered to be different from the other two species and is named *X. neolitseæ*.

Xenostele neolitseæ sp. nov.—Aecia, pycnia, and uredia not known; telia sunk in woody galls formed on leaves and stem, 300–410 μ, with a whitish conical peridium of many layers of sterile, rectangular to polygonal, thick-walled, and warty cells; teliospores two-celled, dark reddish-brown spindle-shaped, sometimes separating into individual cells, 47–0 $\times$ 26·0 μ; pedicelled, pedicels long up to 200 μ, hyaline, fragile.

Habitat.—On living leaves of *Neolitsea zeylanica* Merr. on the Nilgiris, March 1946. Collected by C. L. Subramanian and K. Ramakrishnan (type) at Ootacamund, 15th March 1946. Type deposited in the herbarium of the Government Mycologist, Coimbatore and Herb. Crypt. Ind. Orient., New Delhi.

Aecia, pycnia et uredia non cognita; telia demersa ligneis excrescentiis formatis in foliis et caulibus, 300–410 μ; albo conico peridio multorum stratorum cellarum sterilum, rectangularum ad polygonarum crassomuratorum, echinularum; telio-sporidia bicellata fusci rubricosi brunnei coloris, fusiformia, interdum seperantia in duas cellas, 47 $\times$ 26 μ; Pedicellata, pedicelli longi, hyalini, fragiles, ad 200 μ.

Hab. in vivis folii et rames *Neolitseæ zeylaniceæ* Merr. Ootacamund (Nilgiris) 15-III-1946 C. L. Subramanian et K. Ramakrishnan typus. Typi specimena deposita in Herbario Government Mycologist Coimbatore et Herb. Crypt. Ind. Orient, New Delhi.

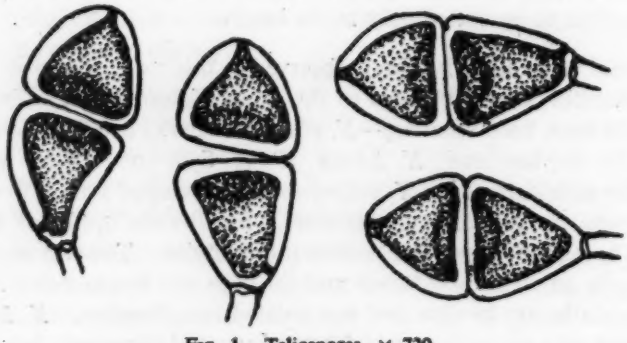

Fig. 1. Teliospores × 720.

(2) *Pseudopeziza rubiæ* sp. nov.

Rubia cordifolia L. a common climber was affected by a leaf spot in the neighbourhood of Lovedale and Coonoor (Nilgiris District of Madras

Province). On the under surface of the spotted region groups of apothecia had developed. Four to five apothecia were present in each group. These apothecia were saucer-shaped 0·5–0·8 mm. in diameter, roughly circular with incurved margins. When fresh the texture of the apothecium is waxy, but it becomes hard and brittle on drying. Young apothecia have a light buff colour but older ones turn dark on the upper surface. The apothecium opens out when mature.

A section of the leaf through the apothecium (Plate, Fig. D) reveals that the latter is sub-epidermal in origin though the whole apothecium is outside the leaf and is carried on a short stalk-like structure 83 μ in length and 125 μ in breadth. This portion broadens out into the hypothecium composed of fairly large, polygonal, thin-walled cells. Above the hypothecium and below the hymenium is the narrow subhymenial layer formed of small-celled tissue. The hymenium is made up of closely packed asci and paraphyses. The asci are hyaline more or less clavate and $70 \times 5{\cdot}2\,\mu$ ($59–93 \times 4–7\,\mu$). The ascospores are eight in number, uniseriate and obliquely arranged. They are hyaline long-oval and $7 \times 2{\cdot}5\,\mu$ ($5{\cdot}5–9 \times 1{\cdot}5–5\,\mu$). The paraphyses are as long as the asci, filiform, unbranched at the tip and hyaline.

P. repanda (Fr.) Karst. has been recorded on the leaves of *Galium* spp. (*G. mollugini, G. borealis, G. silvaticum,* and *G. asperula*) (Butler and Bisby, 1931, and Saccardo, 1889), and leaves and stem of *Rubia tinctorum* (Saccardo, 1889 and 1898) belonging to the Rubiaceæ and *Potentilla cerasti* belonging to the Rosaceæ. On the last host the apothecia are seen on the stems and rarely on the leaves. But the species now recorded is different from *P. repanda.* It has bigger apothecia and larger asci. Further the size, shape and arrangement of the ascospores are entirely different from those of *P. repanda.* Therefore it is named *Pseudopeziza rubiæ.*

Pseudopeziza rubiæ sp. nov. Apothecia hypophyllous, gregarious, light buff when young and dark when old, concave, roughly circular, 0·5–0·8 mm. in diameter; asci, hyaline, long-clavate $70{\cdot}0 \times 5{\cdot}2$ ($59–93 \times 4–7\,\mu$, ascospores uniseriate, hyaline, 8, oblong—$7 \times 2{\cdot}5\,\mu$ ($5–9 \times 1{\cdot}5–5\,\mu$); Paraphyses filiform, unbranched hyaline.

Habitat.—On living leaves of *Rubia cordifolia* L. Lovedale and Coonoor, 19-3-46. Collected by C. L. Subramanian and K. Ramakrishnan. Type deposited in the herbarium of the Government Mycologist, Coimbatore and Herb. Crypt. Ind. Orient, New Delhi.

Apothecia hypophylla, gregaria, levi brunnei coloris in juventu teet fusci cooris in maturitate, concava, cerciter orbicularia 0·5–0·8 mm. nil

diametro; asci hyalini, elongato-clavata, 70·0 × 5·2 (59–93 × 4–7)μ; asco-sporidia uniseriata, hyalina, 8, oblanga 7 × 2·5 (5–9 × 1·5–5)μ; paraphyses filiformes, simplices, hyalini.

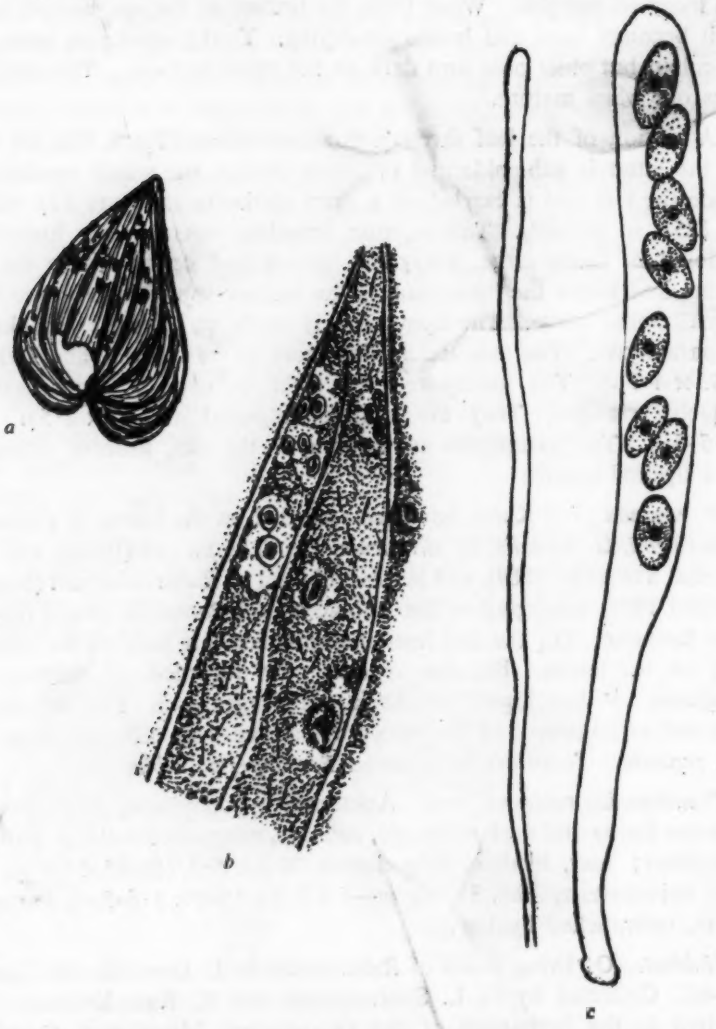

FIG. 2. *a.* leaf with apothecia. *b.* a portion enlarged. *c.* parpaphysis and ascus × 2000.

Habitat in vives foliis Rubiæ cordifoliæ L. Lovedale, Coonoor 19–3–46. Leg. K. Ramakrishnan et C. L. Subramanian. Typi specimina

deposita in Herbario Government Mycologist, Coimbatore et Herb. Crypt. Ind. Orient. New Delhi.

(3) ***Puccinia Linkii*** Koltzch.

This rust was recorded on the leaves of *Viburnum erubescens* Wall.

Telia are epiphyllous and brown spots are visible on the lower surface of the leaves. Very often telia are ring-shaped, erumpent and dark brown in colour. Teliospores are pedicellate, pedicels hyaline. They are two-celled chestnut brown, with hyaline, prominent, sparsely arranged warts on the wall. They measure $42 \times 17{\cdot}5$ ($30{\cdot}75$–$51 \times 10{\cdot}2$–$26{\cdot}7$)μ. They are elliptical with rounded ends, slightly constricted in the middle with one germ pore in each cell—at the apex of the top cell and at the junction of the stalk in the lower cell.

Puccinia Linkii Koltzch has been described (Sydow, 1904) on *Viburnum pauciflorum* in America. The rust on *V. erubescens* resembles that closely and is therefore identified as *P. Linkii*.

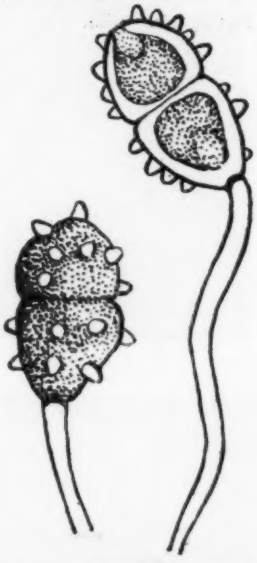

Fig. 3. Telispores (× 720).

The authors acknowledge the help rendered by Mr. M. S. Balakrishnan, Research Fellow, Mycology Section, in making drawings and by Rev. Fr. Singarayar of St. Joseph's Seminary, Coimbatore, in rendering the diagnosis, into Latin. Dr. B. B. Mundkur of New Delhi and Mr. K. M. Thomas,

Government Mycologist, Coimbatore, were kind enough to go through the manuscript and we are grateful to them for their help.

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EXPLANATION OF PLATE

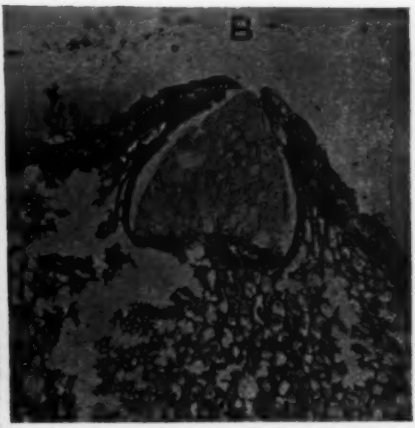
A. *Neolitsea zeylanica* leaf and stem showing the galls produced by the rust (Natural size).

B. Section through the gall showing the telial cup closed by the stopper-like peridium (× 100).

C. A similar section showing the telio-sorus and the teliospores (× 100).

D. Section of apothecium of *Pseudopeziza rubiæ*.

A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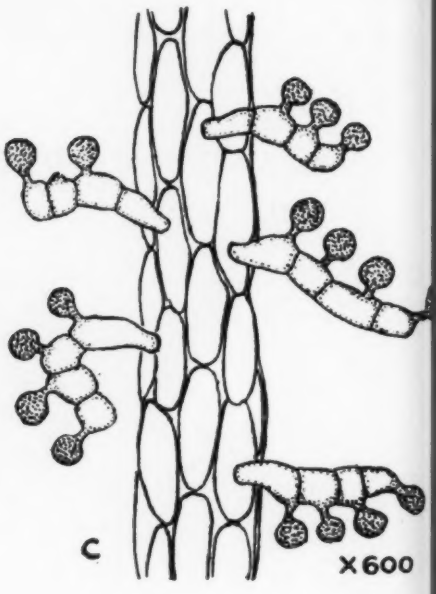

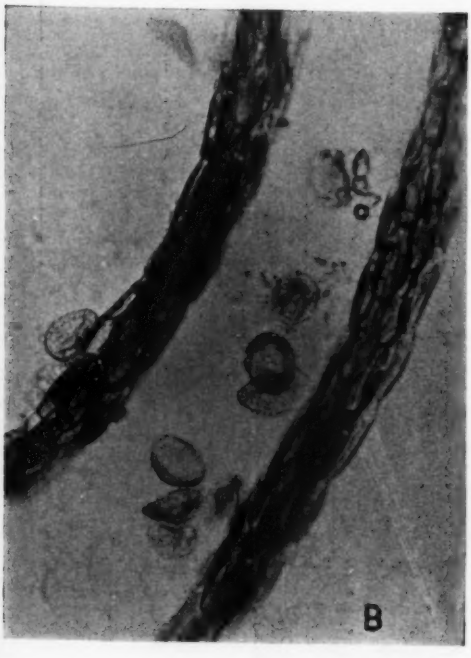

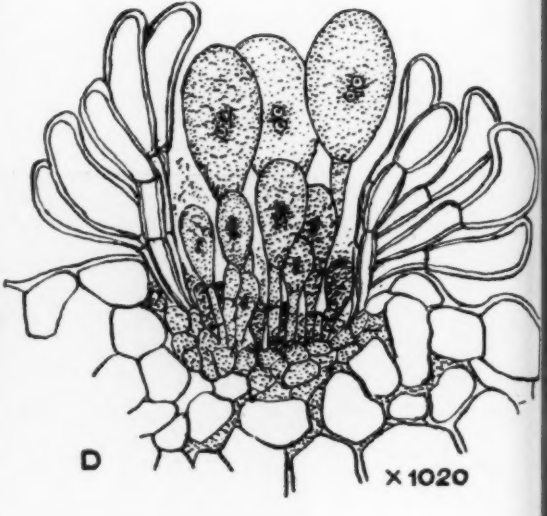

A NEW RUST ON *PREMNA TOMENTOSA* WILLD.

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Received September 11, 1946
(Communicated by Rao Bahadur Dr. B. Viswanath, D.SC., F.R.I.C., C.I.E., F.A.SC.)

Premna tomentosa is common in the foot-hills of the Nilgiri mountains, in the neighbourhood of Kallar (Coimbatore District). In the months of December and January it is affected by a rust.

The uredosori are formed on the lower surface of the leaves. They are minute, crowded, pulverulent, mixed with the tomentum of branched hairs and in mass having a tawny olive colour. The sorus bursts through the epidermis. A peridium is lacking but two or more rows of paraphyses are present all round the periphery of the sorus. These are incurved forming a pseudoperidial structure (Figs. A and D). The paraphyses are one to two septate, with the terminal cell long, often bent and club-shaped. The wall is irregularly thickened and light yellowish brown, rarely hyaline. The uredospores are borne singly on stalks. They are oval or elliptical, prominently echinulate, brownish yellow in colour but with a hyaline spore wall. The spores measure $29{\cdot}5 \times 19{\cdot}5\,\mu$ (the range being $18{\cdot}6 - 31{\cdot}0 \times 15{\cdot}5 - 21{\cdot}7$).

The teleuto-sori are hypophyllous and columnar. The telial columns are solitary but produced near each other. They originate from the sub-epidermal portion and are surrounded by two or more rows of clavate, incurved brown paraphyses as in the case of uredosori. The columns are filiform, tendril-like and many of them are intertwined. Each column is about 5–6 mm. in length and 25–35 μ in thickness made up of 5–7 rows of closely united cells. The teleutospores are one-celled, sessile, oblong, yellow-ochre in colour and measure $28{\cdot}5 \times 8{\cdot}7\,\mu$ (range being $17{\cdot}1–44{\cdot}9 \times 4{\cdot}7 – 12{\cdot}4$). All the spores are closely united together (Plate V, Fig. B).

The teleutospore is capable of immediate germination. When portions of the telial columns are floated on drops of water kept on a slide inside a moist chamber, germination takes place in 8–10 hours at room temperature (28° C.). A stout, 4-celled basidium grows out of the spore. From each

cell a short sterigma develops and on this a hyaline round or oval basidiospore is formed (Plate V, Fig. C).

Petch (1911) has described *Cronartium premnæ* on *Premna corymbosa* R. and Willd. from Ceylon. Sydow (1918) amended this as *Crossopsora premnæ* (Petch) Syd. The uredospores of this fungus are stated to be 20–28 by 16–19 μ; thick-walled, hyaline clavate paraphyses are present in the uredosorus. The teleutosori are several millimetres in length and about 50 μ in diameter and the spores are 40–58 $\times$ 8 μ.

The rust on *Premna tomentosa*, however, belongs to the genus *Crossopsora*. Since the uredosorus has no peridium but has a ring of incurved paraphyses round it, this rust cannot be a *Cronartium* but only *Crossopsora*.

The rust on *Premna tomentosa* differs from that recorded on *P. corymbosa* in having thinner telial columns and smaller teleutospores. Since it has not been recorded before and is new it is described as *Crossopsora premnæ-tomentosæ*.

Crossopsora premnæ-tomentosæ sp. nov.—Uredosorus hypophyllous, minute, crowded, erumpent, pulverulent, with a ring of incurved, 1–2 septate light-brown paraphyses; uredospores oval to elliptic, echinulate, contents brownish yellow, wall hyaline, 29·5 $\times$ 19 μ; teleutosorus hypophyllous, filiform, surrounded by a ring of several rows of paraphyses at the base, dark brown, 5–6 mm. in length, 25–35 μ in diameter, teleutospores sessile, one-celled, united, oblong, yellow-ochre in colour 28·5 $\times$ 8·7 μ.

Habitat.—In living leaves of *Premna tomentosa* Willd. at Kallar, Coimbatore District, January 6th, 1946 (Soumini and Krishnamurthy). Type specimen deposited in the herbarium of the Government Mycologist, Coimbatore.

Crossopsora premnæ-tomentosæ.—Uredosoris hypophyllis, minutis, gregariis, erumpens; pulverulentus, paraphysibus, numerosus, 1–2 septatis, introrsum curvatus, levi brunneis; uredosporis, ovatis, v. ellipsoides, echinulatis, flavo brunneis, 29·5 $\times$ 19 μ; episporio hyalinis; teleutosoris, hypophyllis, filiformibus, circumdatum annulo gradum multorum paraphysium basi, fuscum, 5–6 mm. long, 25–35 μ lat.; teleutosporis arcteconnexis, oblongatis, 28·5 $\times$ 8·7 μ, flavus ochraceus.

Hab. in vivis foliis *Premnæ tomentosæ* Willd. Kallar, Coimbatore District, 6-1-1946 (Soumini and Krishnamurthy).

We are indebted to Mr. C. S. Krishnamurthy for helping in the collection of specimens, to Mr. S. N. Chandrasekhara Ayyar for the identification of the host and to Rev. Fr. Singarayar of St. Joseph's Seminary, Coimbatore, for the Latin translation of the diagnosis.

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Explanation of Plate

A. Photomicrograph of a uredosorus (× 400).

B. Photomicrograph showing portions of telial columns and some uredospores (× 400).

C. Germination of teleutospores (× 600).

D. Drawing of a section through a uredosorus (× 1020).

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FRUIT ROT OF TOMATOES CAUSED BY *PHYTOPHTHORA PALMIVORA* BUTL.

By T. S. Ramakrishnan and C. K. Soumini
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Received June 15, 1946
(Communicated by Dr. B. Viswanath, C.I.E., D.SC., F.R.I.C.)

During the north-east monsoon period in 1944 and 1945, a fruit rot disease of tomatoes was in evidence at the vegetable production centre at Tudiyalur, Coimbatore district. The crop was raised in a field where the plants were not propped. The disease became evident soon after heavy rains in October. Since the propping was not done several branches were spreading on the ground and consequently some of the fruits borne on these branches were at times in contact with the wet soil. Such fruits were the first to be affected. Nearly 25 per cent. of the fruits were involved. The disease later on spread to fruits borne on higher branches also.

The disease was observed mainly on the fruits. In a few instances the young shoots touching the soil were also affected. The stem and the branches at this region were first discoloured with a dull green water-soaked appearance but later these turned dark brown and rotted. Fruits of all sizes were affected. On green fruits the disease commences at the blossom end or at the side which touches the soil in the form of small water-soaked spots. These increase rapidly in size and in the course of 3 to 4 days the entire fruit becomes involved. The fruit assumes a brownish colour, is soft to the touch and the skin easily peels off. In wet weather the fungus grows out and forms a white fluffy growth on the surface. Sometimes, concentric markings may be seen in the affected portions and the external fungal growth also assumes similar distribution (see photograph). The organism causing the disease was found to be a *Phytophthora* and numerous sporangia were detected in scrapings of the external growth.

Fruit rots of tomato caused by *Phytophthora* have been recorded from all over the world. Tucker (1933) has recorded a rot of fruits near or in contact with soil as the most common type of infection caused by *Phytophthora parasitica* Dast. Reddick (1920) has described the occurrence of a disease in glass houses in New York causing girdling of the stem and rapid rotting of fruits due to *P. parasitica* Dast. *P. infestans* de Bary has been known to infect fruits in various countries (Tucker, 1933). Buckeye rot of

tomatoes in California is ascribed to *P. dreschsleri* Tucker and *P. capsici* Leonian (Tompkins and Tucker, 1941). Lavellée (1941) has recorded *P. parasitica* Dast. as responsible for buckeye rot. Thus tomato fruit rot is widespread and is reported to be caused by different species of *Phytophthora*.

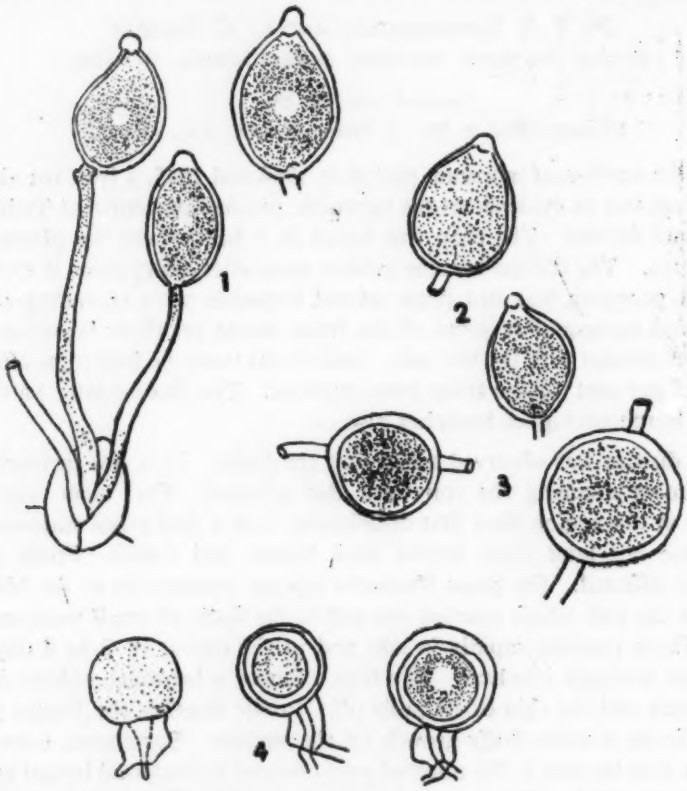

Fig. 1. Sporangia from tomato fruits (×400).
Fig. 2. Sporangia from culture (×400).
Fig. 3. Chlamydospores from culture (×400).
Fig. 4. Oospores formed in combination with another strain (×400).

The organism causing the disease at Coimbatore was isolated into pure culture from a single sporangium. It grew well on oat and french-bean agar media. Numerous characteristic sporangia and chlamydospores were produced. The sporangia are oval or pear-shaped, papillate, mainly terminal and measure 33·4 × 22·6 μ (18·6–46·5 × 15·5–37·2 μ) (Figs. 1 and 2). The chlamydospores are spherical, hyaline or light yellowish brown in

colour. The colour is developed in the wall of the older chlamydospores. They were formed terminally or more often intercalary and measured 23·4 × 20·5 μ (15·5–31 μ) (Fig. 3). Oospores were not formed.

The pathogenicity of the organism was established by inoculation experiments of the fruits on growing plants and detached fruits kept in sterilized moist chambers. The plants with the fruits (grown in pots) were placed in glazed cages kept moist by having a layer of moist sand at the bottom and frequent sprayings with sterile distilled water. The fruits were young and green. All the inoculated fruits were infected and in 5 days they rotted completely. The detached fruits were green, bigger and more mature. These were infected in 3 days and completely rotted in 6 days. In both cases the same fungus was recovered from the infected fruits. The controls remained healthy throughout.

The organism is able to infect young branches and leaves of tomato. These become involved in a blackish green wet rot and the rotten portions fall off or the stem breaks at the point of infection. With the severance of the infected branch or stem the spread of infection is arrested.

The organism infects the fruits through unwounded surfaces. Inoculation experiments showed that infection can take place through any part of the fruit. Bits of culture were placed on the fruit near the stalk, stylar end and other portions of the fruit and in all cases infection occurred. The hyphæ ramify through the tissues of the fruit being both inter-cellular and intra-cellular. The affected tissues became soft and discoloured.

The average dimensions of the sporangia and chlamydospores of this *Phytophthora* agree with those of *P. arecæ* (Coleman) Pethybridge, *P. palmivora* Butler, *P. meadii* Mc Rae, and *P. parasitica* Dastur. Tucker (1931) is of opinion that "the dimensions of sporangia considered independently of other characters cannot be accorded much importance taxonomically". The same may be said to apply to the dimensions of chlamydospores also.

The fungus was grown in paired culture with two strains of *Phytophthora* isolated from arecanut and kindly supplied by Dr. Uppal, Plant Pathologist, Bombay. Oospores were produced with one of these strains but not with the other. The oospores were spherical yellowish in colour and measured 20·0 μ in diameter (range 15·5–24·8 μ) (Fig. 4).

The isolate from tomato closely resembles one of the strains isolated by Uppal and Desai (1939) from arecanuts (Nilekani strain) and is similar to the strain found on arecanut in South Kanara. A more detailed study including all the South Indian isolates of *Phytophthora* and others obtained

from elsewhere is being carried out in this laboratory and will form the subject of a further communication.

Control

Experience in other parts of the world has shown that when the tomato plants are sprayed with Bordeaux mixture the fruit rot disease is controlled. Further it is noticed that the disease commences on the fruits lying in contact with the soil. If it is possible to prevent this, the incidence of the disease can be lowered. This can be accomplished by staking the plants or tying them to frames and thus prevent them from trailing on the ground. Further the plants and fruits must be sprayed with Bordeaux mixture.

Summary

A fruit rot of tomatoes was prevalent in Coimbatore during the rainy season. Fruits in contact with the soil were the first to be affected. *Phytophthora* was isolated from these fruits. The fungus was found to resemble *P. palmivora* Butl. (areca strain from S. Kanara).

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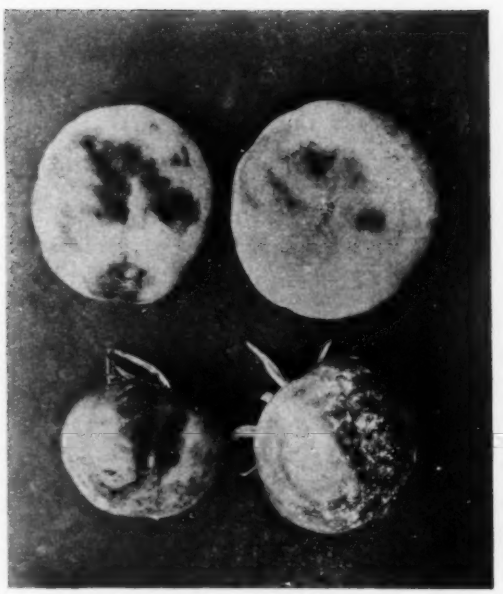

Diseased tomato fruits

CALIGUS SCIAENAE N. SP. PARASITIC ON *SCIAENA GLAUCA* FROM MADRAS

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Received January 13, 1947
(Communicated by Prof. S. G. M. Ramanujam, F.A.Sc.)

As against the exhaustive studies of British parasitic copepods by Scott,[7] of American forms by Wilson[9] and European species by Brian[2b] and Hansen,[4] our knowledge of copepods parasitic on Indian fishes is poor. Bassett Smith,[1] Kirtisinghe,[5] Thompson,[8] Wilson[9] and Brian[2a] and Grey have recorded and described a few forms. Hence a full description of this parasitic caligid copepod was deemed not superfluous.

This parasite was found attached to the tip of a gill filament of the first gill of *Sciæna glauca*. It measures 1·7 mm., the setæ of the anal plates included. (The frontal area is 1 mm.; the cephalothorax ·7 mm.; abdomen ·7 mm.; the anal plates and setæ ·2 mm.). The frontal region is marked by the possession of two large lunules visible even dorsally. Examined ventrally each sucker appears like a deep spherical cup. The rim of the cup is turned in to form a flat shelf. This edge of the sucker is not entire, being cut up anteriorly and the two cut ends overlapping each other to a slight extent. The entire lunule is clearly formed by the folding of the edge of the frontal plate. Medially the anterior border of the frontal area shows a projection on the ventral side. This projection is a median sucker-like fold of the frontal edge and occurs just where the frontal filament would have been during the Chalimus period in the development. The persistence of this sucker along with the well-developed lunule shows that it has just passed the Chalimus stage. The frontal area is also marked by the occurence of the 1st antennæ whose basal joint appears continuous with it.

The cephalothorax is almost circular in shape being ·7 mm. long and ·75 mm. broad (Fig. 1). The cephalic area is marked off from the thoracic area by a semi-circular groove. The carapace as well as the rest of the body appears whitish, transparent and free from colour marks. The posterior edge of the cephalothorax area extends dorsally over the free thoracic segment which is clearly visible from the ventral aspect. With the convex form of the cephalothoracic shield and the flattened or slightly hollowed form of the sternal plate of the third thoracic segment, a cupping adhesion

can be effected by the body of the parasite whenever necessary. The fact that the cephalothorax is formed of ten segments (the seven-segmented cephalon as well as three segments of the thorax united with it) is obvious from the ten pairs of appendages found on the ventral side. On the dorsal side the double median eye can be made out.

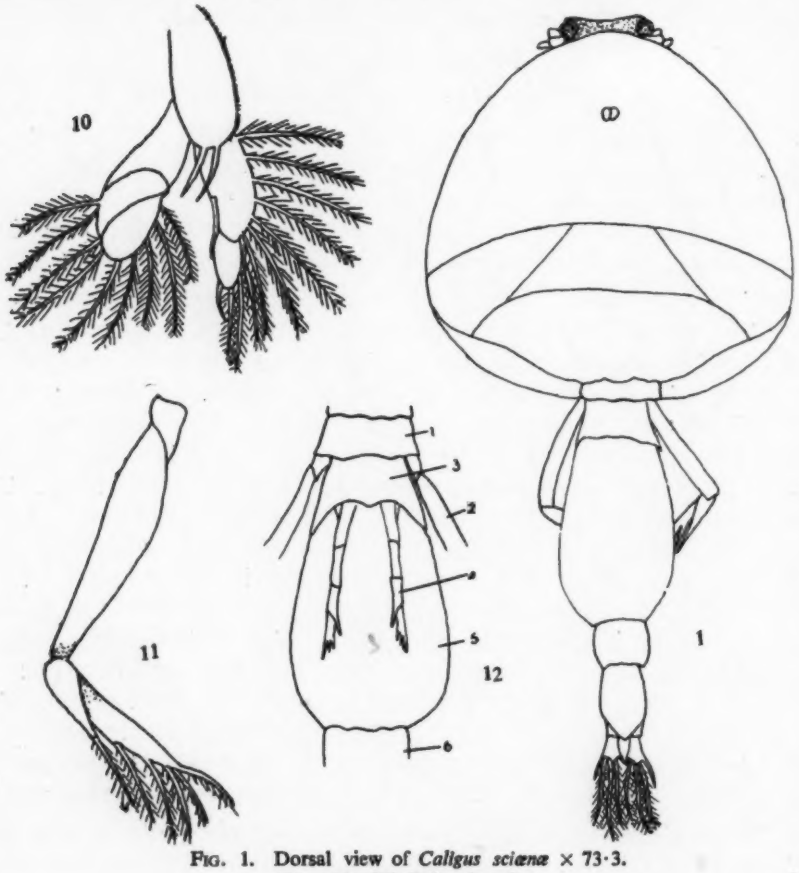

Fig. 1. Dorsal view of *Caligus sciænæ* × 73·3.
Fig. 10. III Swimming leg.
Fig. 11. IV Swimming leg.
Fig. 12. V Thoracic legs and the genital segment:
1. Free thoracic segment. 4. Vestigial fifth leg.
2. Fourth swimming leg. 5. Genital segment.
3. Fifth thoracic segment. 6. Second abdominal segment.

1*st Antenna* (Fig. 2) is three-jointed, the basal joint being as broad as the lunule itself. It is heavily armed with about fifteen stout spines while

the distal joint is long, slender and bears two spines on its body and four spines at its distal end.

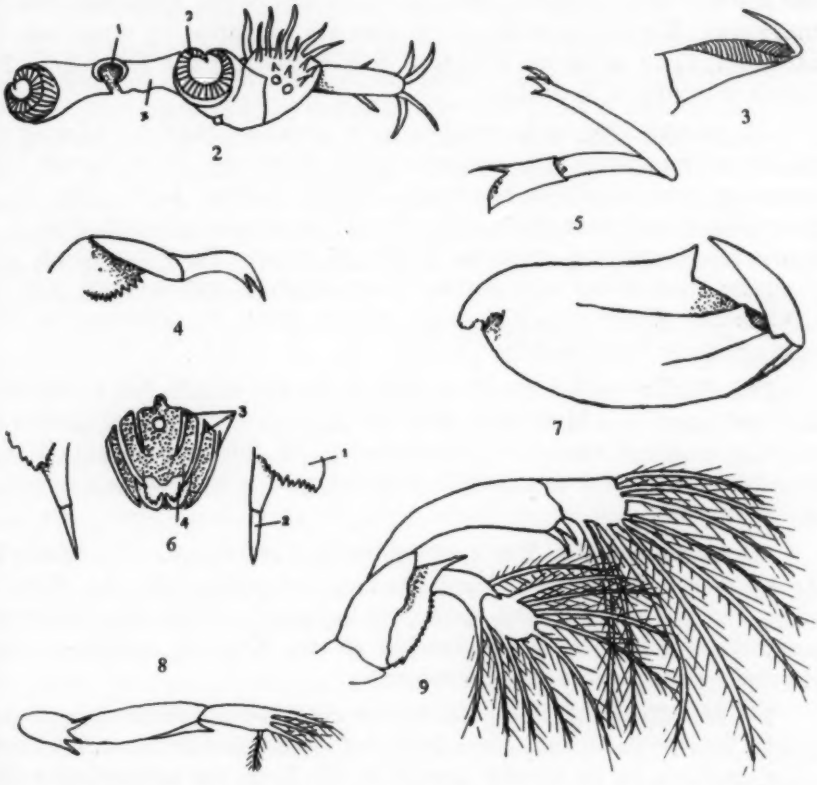

FIG. 2. Frontal Plate and Antenna: 1. median sucker. 2. lunule. 3. frontal plate.

FIG. 3. II Antenna.

FIG. 4. I maxilla.

FIG. 5. I Maxillipede.

FIG. 6. II Maxillae and mouth tube:

1. Exopod of maxilla.
2. Endopod of maxilla.
3. Chitinous rods of mouth tube.
4. Mandible.

FIG. 7. II Maxillipede.

FIG. 8. I Swimming leg.

FIG. 9. II Swimming leg.

2nd Antenna (Fig. 3) is two-jointed, the proximal joint being stouter than the distal, which is long, slender and terminates in a sharp curved spine. On the inner side are seen two grooved plates.

Mandible is long and slender, toothed like a saw with the distal tip curved inwards. The mandibles are connected with the mouth tube, in the posterior part of which the teeth at the tips of the mandibles can be made out. The lateral walls of the tube are supported by three rods on each side, there being no transverse rods supporting the lower lip. The mouth is reniform in outline.

1*st Maxilla* (Fig. 4) is attached more outwards than the base of the second antenna but a little posterior to the front edge of the mouth tube containing the mandibles. It consists of two distinct joints. The basal joint is short and stout made stouter by the occurrence of toothed plate or lamina representing the exopodite or palp (F. Scott). The distal joint is very decidedly hooked and with its two sharp recurved spines must help in attachment. Wilson described only a simple claw; the occurrence of two in the parasite is noteworthy.

2*nd Maxilla* which is nearly as long as the first maxilla has a columnar base and tapers to a blunt point (Fig. 6). A small many-toothed lamina at the base probably represents the exopodite of this appendage (Wilson describes two setæ and considers them as endopodites and the main structure as the exopod).

1*st Maxillipede* (Fig. 5) is a very prominent appendage. It is distinctly three-jointed. The first two joints are long and stout while the third is longer and more slender, and appears to be capable of considerable movement. It ends in three sharp claw-like spines. There is a single-toothed plate at the base, indicating the endopod.

2*nd Maxillipede* (Fig. 7) arises nearer the mid-line and consists of two distinct joints. The basal joint is large and swollen and flattened. It bears a distinct tooth on its anterior border in this form, the terminal claw-like part folding like a knife-blade. At the outer part of the stout basal joint where the rest of the limb folds back can be seen a toothed bony plate rising from the posterior border of the base. At the very bottom of the basal joint on the medial aspect can be seen another toothed plate, not unlike those described before in the maxillæ. As this occurs on the medial aspect it is probably homologous to the endopod.

1*st swimming leg* (Fig. 8) as well as the second and third swimming legs indicate the thoracic segments which have united with the cephalon. The first leg however is uniramous like the fourth leg. It is three-jointed. The basal joint is short and stout. Its outer margin bears three sharp spines while the fourth spine borne by the body projects tailward. The second joint is longer by half the length of the first and bears a spine at its outer

margin. The distal joint bears three straight spines poirting outwards and a fourth spine directed backward. These spines are provided with fine hairs. The second and third joints represent the exopod.

2nd swimming leg (Fig. 9) has a short stout basal joint bearing both the exopod and the endopod. The inner ramus of the limb is three-jointed. The first joint has a toothed posterior edge and bears a spine at its outer margin; the second joint is shorter and bears a tooth at its outer edge while the third is flat, broad and orbicular. It is fringed with nearly ten long plumose setæ. The exopod is longer chiefly due to the first two joints which are similar to those of the endopod. The third joint bears on its posterior edge about the middle of its length, two short spines and at its distal margin bears eight long plumose setæ.

3rd swimming leg (Fig. 10) is attached farther from the mid-line because of the enlargement of the sternal plate of the segment. The natatory function of this appendage is unmistakable for even the basal segment is broad and flat. It is fringed on the outside by numerous short hairs and also bears two long plumose setæ on the ventral side of the outer edge. The endopod is foliaceous and three-jointed. The outer two joints bear nearly ten long plumose setæ. The exopod is also three-jointed, the outer two joints bear nearly ten long plumose setæ.

4th swimming leg (Fig. 11) is uniramous, the endopod being absent. It is four-jointed. The basal segment is stout as in the other legs. The first joint of the exopod is by far the longest forming nearly half the length of the limb. The second joint is short but is produced into a long spine at its outer margins while the third is slender bearing four apically directed spines with a fifth spine at the distal end. All the spines or setæ are plumose.

5th swimming leg (Fig. 12) represents a fifth thoracic segment as pointed out by Wilson. This occurs on the ventral side of the "genital segment". But as can be seen in the form described in this paper, these appendages really spring from the front part of the "genital segment". This anterior part of the segment is separated from the genital segment proper by a distinct groove. Therefore the genital segment is regarded as really the 1st abdominal and not as the fifth thoracic segment as Wilson has done.

The abdomen is three-jointed. The genital segment is nearly twice as broad as the succeeding segment, and nearly four times as long. There are no vestiges of appendages or other indications to show that this genital segment may be a composite of two segments fused into one. The segment behind it is broader than long while the third and last segment is longer than

broad. It ends in an obtuse point on either side of which the anal plates occur. Each lamina bears three long plumose setæ on its posterior side and two stouter spines one at each of the posterior corners. The absence of special structures on the antenna and of special plates on the 1st maxillipede usually used for prehension by the male, make it probable that the parasite is a female. The size of the genital segment also does not contradict such a conclusion. The persistence of the median sucker (the relic of the frontal filament of the Chalimus stage in development) as well as the absence of any trace of the egg strings, both argue the immature condition of the subject. This also serves to explain the occurrence of the fifth pair of thoracic legs (though in an unusual condition and position, being pressed against the body) whereas these usually disappear in a mature female. The occurrence of this species of *Caligus* on *Sciæna glauca* of the Madras Coast is noteworthy since *Caligus* (*Sciænophilus* Van Beneden) *Benedeni* sp. nov. described by Bassett Smith,[1] was taken from *Sciæna diacanthus* from Bombay and later recorded from Ceylon by Thompson.[2] This species, *C. Benedeni*, differs from the form described in this paper in having the cephalothorax only a fifth of the whole length and being much less broad than the genital segment; the lunules being very small; the basal part of the 1st antenna having only twelve plumose setæ and the second joint having two long spines; the 1st pereiopod having three long end bristles and three moderately long plumose setæ on its posterior border; the genital segment being rather long than broad; and the abdomen being single-jointed. In view of these differences the present form is described as *Caligus sciænæ*, n. sp. in this paper.

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(I) THE INTERACTION BETWEEN IONS DRUGS AND ELECTRICAL STIMULATION AS INDICATED BY THE CONTRACTION OF AVIAN UNSTRIATED MUSCLE. (II) ACTIVE ELONGATION OF UNSTRIATED MUSCLE

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Received December 11, 1946

The experiment on *Mytilus*, frog and mammalian muscles have been continued on the unstriated muscle from the domestic fowl to see if any differences exist. The body temperature of the birds is slightly higher than that of mammals and this might result in certain reactions. In mammals the differences between the responses of their unstriated muscle from frog unstriated muscle may be ascribed to: (1) greater ionic content of the saline, (2) to greater body temperature. The latter results in slower adaptation which increases sensitivity, while the former produces opposite results.

Experimental

The saline used was as previously (Singh, 1939). The experiments were performed at room temperature (25–30° C.) as this range is optimum for most reactions. The muscles used were the duodenum and the œsophagus of the domestic fowl. The duodenum forms a U-loop enclosing the pancreas, blood vessels and nerves. One limb of the U-loop may be taken out with or without the attached pancreas and nerves; it provides a straight portion of the gut. The œsophagus was chosen, as its responses were exactly like those of the other unstriated muscle; a muscle nerve preparation was made.

Results

The reactions of avian unstriated muscle resemble those of the other unstriated muscle, with the exception of a few differences.

Effect of temperature.—The optimum tempearture for alternating current for the duodenum is 29–30° C. and for the œsophagus, 25–26° C. This lower temperature for the œsophagus is presumably due to its exposed position (Singh, Singh and Muthana, 1946). In the dog stomach, the optimum temperature at Bombay, which has similar climate as Karachi, was 24–25° C.

The higher optimum temperature in the fowl is presumably related to its higher body temperature. The optimum temperature for potassium is 20° C. and for acetylcholine is 25° C., in the dog the optimum temperature for acetylcholine being 30° C. The optimum temperature is however variable (Singh and Rao, 1940). This appears to be due to adaptation, Fig. 1 (Singh, 1946). Tone may increase or decrease at high temperatures

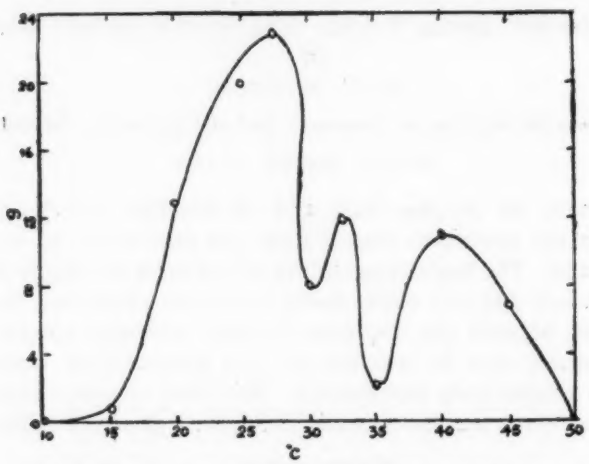

FIG. 1. *Fowl Duodenum.*—The effect of temperature on A. C. contraction

(35–39° C.). The increase resembles as in other unstriated muscle, and the decrease is probably due to adaptation, which increases at higher temperatures (Singh, 1940). The duodenum is more affected by cold than other unstriated muscles, a few degrees increase in temperature above 25° C. may produce great increase in excitability to alternating current.

Effect of osmotic pressure.—The increase in osmotic pressure of the saline by addition of sucrose or sodium chloride beyord 20 to 40% above normal is depressant. The results of increase up to 20% are variable. In the œsophagus increase in osmotic pressure by addition of sucrose to the saline increases the response to alternating current, decreases tone and the response to acetylcholine and potassium. With sodium chloride the response to alternating current decreases and that to potassium and acetylcholine is variable. In the duodenum, with sucrose, the response to alternating current increases, tone decreases, the response to acetylcholine increases and to potassium is variable. These effects are similar to those in *Mytelus* muscle.

Decrease in osmotic pressure of the saline by 20% in the œsophagus increases the response to alternating current, decreases tone and the response to potassium and acetylcholine; in the duodenum the response to alternating and tone is increased, and to potassium and acetylcholine is decreased. Further reduction in osmotic pressure is depressant to all in the œsophagus as well as duodenum, though tone in the latter may increase.

Effect of calcium.—In the œsophagus the optimum concentration of calcium for alternating current, acetyl choline, potassium, and nervous stimulation is 0·00206 *M* $CaCl_2$, though the œsophagus may become hyperirritable in the absence of calcium. In the duodenum, the optimum concentration for alternating current is three to four times that in the œsophagus but for potassium and acetylcholine it is the same. As in the dog stomach excess of calcium up to 0·02 *M* $CaCl_2$, both in the duodenum as well as œsophagus may potentiate the response to acetylcholine and potassium.

Strontium acts like calcium; barium produces tonic contraction and so causes depression of excitability. Magnesium is depressant.

Effect of lithium.—Replacement of sodium of the saline with lithium produces effects of sodium deficiency. Replacement of 20–40% of the sodium increases the response to alternating current, potassium and acetylcholine, tone decreases.

Effect of sodium.—Replacement of part of the sodium chloride (20–40%) increase the response to alternating current, potassium and acetylcholine. Further increase is depressant. Complete removal of sodium chloride causes contraction; so also isotonic sucrose, suggesting that difference in ionic concentration on two sides of the membrane causes contraction.

Effect of ammonium.—The replacement of the 20% of sodium of the saline with ammonium decreases the response to alternating current but increases that to potassium and acetylcholine. Further increase is depressant. Ammonium thus potentiates the response to potassium and acetylcholine, and may cause contraction. Withdrawal of ammonium may cause contraction.

Effect of potassium.—The optimum concentration of potassium for the response to alternating current, potassium and acetylcholine is 0·0016 *M* KCl. Further increase is depressant and causes tonic contraction. The gut muscle is rather sensitive to potassium.

Effect of hydrogen ions.—The optimum pH for alternating current in the duodenum is 8; at pH 6 it may become inexcitable. In the œsophagus this is also the optimum, but it may cause depression owing to increase of

tone, then the excitability declines as the pH is decreased from 9·24 to 8 and then increases up to pH 7 (Fig. 2). Tone decreases with increase in hydrogen ion concentration. Increase in hydrogen ions do not potentiate the response to potassium and acetylcholine.

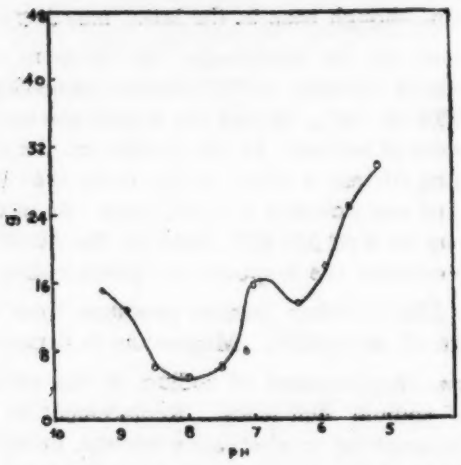

FIG. 2. *Fowl Oesophagus.*—The effect of pH on A. C. contraction

Effect of anions.—Small concentrations of Br, NO_3, I, SCN increase and large concentrations decrease the excitability, when they cause tonic concentration.

Effect of eserine.—There is a marked difference between the reactions of the fowl gut and œsophagus on one hand and the dog stomach on the other hand. It does not cause marked increase in excitability in the former as it does in the latter; it rather causes depression. It does not potentiate the response to acetylcholine; if at all, the potentiation is insignificant (*cf.* Brown and Harvey, 1938). It may cause depression. 1 in 10^7 potentiates the response to alternating current.

Effect of adrenaline.—It depresses the response to alternating current in concentrations of 1 m 10^6 or greater. Lesser concentrations have insignificant effect. It increases the response to potassium and acetylcholine in concentrations 1 in 10^7–10^5.

Effect of acetylcholine.—1 in 10^9–10^4 potentiates the response to alternating current. It causes depression if it produces tonic contraction.

Effect of nerves.—For some unknown reason, the muscles after one or two responses become inexcitable to nervous stimulation. The œsophagus

may become inexcitable to acetylcholine and potassium as well. The response to alternating current, however, remained. In such muscles the responses to alternating current did not differ significantly from other muscles. So it appears that alternating current produces its effect by direct stimulation of the muscle, though the latter may also be stimulated through its nerves. It is possible, however, that the inexcitability may be due to lack of conduction in the nerve (Bülbing and Burn, 1939) but adrenaline did not restore the responses. Potassium stimulated the duodenum which was inexcitable to nervous stimulation; so it directly acts on muscle (Singh and Muthana, 1946). Excess of calcium may make the muscle inexcitable to nervous stimulation, but hypersensitive to acetylcholine.

Active Elongation

An interesting feature was noticed that in sodium deficient solutions the muscle elongated when stimulated with alternating current. This was produced as follows: An isometric lever was used. The hook at the bottom of the muscle chamber reached up to the narrow part of the chamber. The muscle was directly tied to the hook, and it rested on the hook; it was put under slight tension of about 5–10 g. If a part of the sodium chloride (20–60%) of the saline was replaced with sucrose or lithium chloride, the muscle elongated when stimulated with alternating current (8 volts). The elongation was continuation of relaxation after contraction. It occurred in 3 out of 55 experiments in duodenum in sucrose saline and in one out of 6 experiments in lithium saline, and was once observed in rabbit gut in ordinary saline. It has never been produced if the muscle was placed in a trough isotonically and then stimulated. The conditions for its occurrence are not understood. It appears that some initial tension is necessary.

The elongation may be due to two causes: (1) contraction of circular muscle, (2) active elongation of longitudinal fibres. The latter is the correct explanation, as there was no evidence of marked contraction of circular muscle throughout the gut. In one experiment it was relaxed throughout. In sodium deficient solutions, tone decreases, so that it appears that elongation is an active process, as it is in skeletal muscle (McDowall, 1944 ; Lloyd, 1946). The fact that it occurs in sodium deficient solutions supports the view that the latter may be responsible for tonus (Singh and Singh, 1946).

Summary

1. The responses of avian plain muscle in general resemble those of mammalian plain muscle.

2. Eserine has little or no potentiating effect on the action of acetylcholine.

3. In sodium deficient solutions, the gut elongates activity when stimulated with alternating current.

4. In a muscle inexcitable to nervous stimulation, alternating current produces its usual effects.

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CONTRIBUTIONS TO THE BIONOMICS, ANATOMY, REPRODUCTION AND DEVELOPMENT OF THE INDIAN HOUSE-GECKO, *HEMIDACTYLUS FLAVIVIRIDIS* RÜPPEL

Part IV. The Respiratory and Vocal Organs

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1. INTRODUCTION

THE Reptilian respiratory system has already been investigated in great detail from several standpoints—anatomical, histological, developmental and functional. In the last century, most of the workers were naturally interested in morphological and embryological studies, while in the present one there is a perceptible preference for physiological investigations.

The anatomy and histology of the system has attracted a host of investigators since the first quarter of the nineteenth century. Tiedemann (1818), Meckel (1818), Schulze (1871), Leydig (1872), Jullien (1878) and Milani (1894 and 1897) studied the minute structure of the trachea and lungs; while Owen (1866), Hoffmann (1890), Gegenbaur (1899) and Wiedersheim (1890) gave a general survey of the results so achieved. Wiedersheim

(1906) described the respiratory organs of the geckos *Phyllodactylus* and *Platydactylus*; and Werner (1911) those of certain rare reptiles. Ábrahám (1911) investigated the nerve endings in a saurian lung; Gräper (1929) the closure of pleural cavities and the differentiation of the lung in reptiles; and Baudrimont (1929) the muscular and elastic fibres in it. Rothley (1930) dealt with the minute structure of the trachea and lungs, and Smirnowsky (1930) and Dombrowski (1930) with the respiratory musculature.

The development of the lung in reptiles was worked out by Moser (1902), Hesser (1905), Bertelli (1905) and Heilmann (1914), and that of the trachea in *Lacerta agilis* by Böker (1917–18).

The physiology of the system has been the subject of numerous detailed investigations in the present century. Tornier (1904) studied the structure and function of the cervical air sacs and valves in Chameleon, and Couvreur and Gautier (1904) the respiratory rhythm in it. Rainaldi (1907) described the respiratory apparatus of *Lacerta muralis*. Francois-Franck investigated the contractility and innervation of lungs in the ocellate lizard (1907) and the mechanism of respiration in *Chameleon vulgaris* (1907), Grecian Tortoise (1908), and the ocellated lizard (1907 and 1909). Baglioni (1911) gave a résumé of the comparative physiology of lung movements in Amphibia, Reptiles, Birds and Mammals. Babàk (1914) dealt with the lung movements and their regulation in Lizards; Milligan (1924) with the respiration and metabolism in *Sphenodon*: Potter and Glass (1931) with the respiration in the hibernating *Phrynosoma cornutum*, and Wolf (1933) with the structure and function of reptilian lungs. Gnanamuthu (1933 and 1937) tried to correlate the movements of the buccal floor with those of the thorax in lizards and turtles; while Saalfeld (1933) investigated the nerve regulation of lung movements in *Uromastix*.

The larynx of reptiles was investigated as early as 1839 by Henle in the course of his classical researches on the comparative anatomy of this organ in vertebrates. Dubois (1886), Wilder (1892), and Göppert (1899) discussed its homology; Wiedersheim (1906) described it in *Platydactylus mauritanicus*; Germershausen (1913) investigated it in *Chameleon*; while Schmidt (1913) studied its development in reptiles.

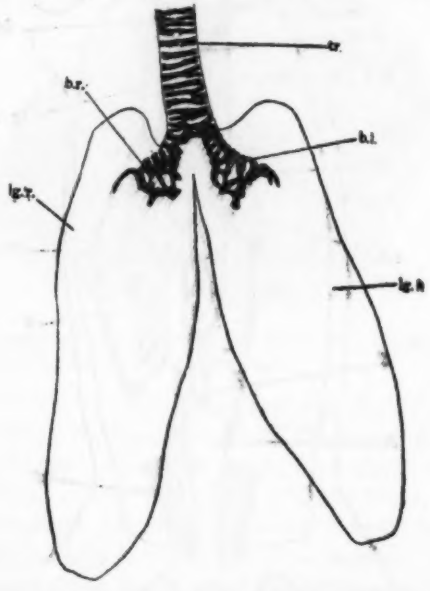
2. Technique

In order to make out the relative position of the various organs in the thoracic part of the perivisceral cavity, as well as to study the disposition of the peritoneum, thick hand-cut transverse sections of formaldehyde-preserved specimens, sagittal sections, and careful dissections from the right

or left side proved useful. The structure of the lungs was studied by staining small pieces in borax carmine and mounting them in balsam, as well as by preparing transverse and longitudinal sections. The skeleton of the larynx, trachea, bronchi and hyoid apparatus was investigated in entire mounts, stained either according to Dawson's Alizarin Red method, or by Van Wijhe's method of cartilage staining. The soft parts of the vocal apparatus were studied in series of transverse and longitudinal sections 10 microns thick, stained by Mallory's triple stain, as modified by Krichesky [*Stain Technology*, VI (1931), 97].

3. The Respiratory System

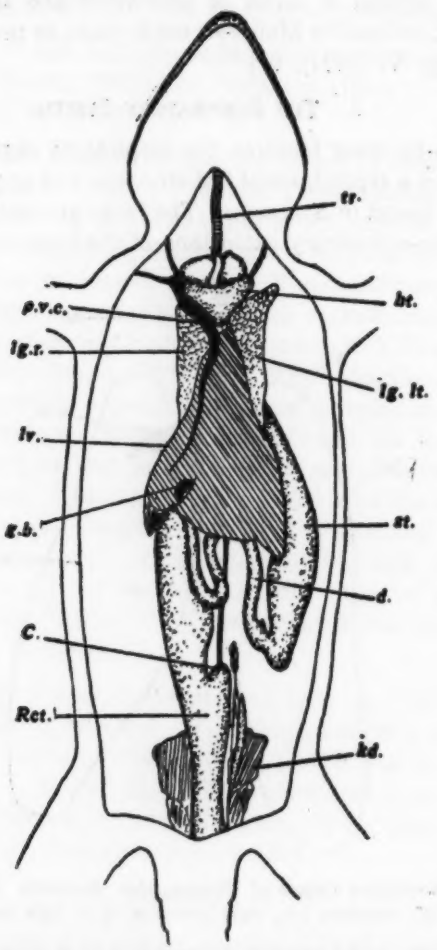
(*a*) *General.*—In many features, the respiratory organs of *Hemidactylus* (Text-Fig. 1) show a typical, simplified structure and appear to approximate to the condition found in *Sphenodon*. The lungs are equal and symmetrical; there are no intra-pulmonary extensions of the bronchi; the lumen of the



Text-fig. 1. Respiratory Organs of *Hemidactylus flaviviridis* (from a cartilage-stained preparation).—*b.l.*, left bronchus; *b.r.*, right bronchus; *lg.r.*, right lung; *tr.*, trachea.

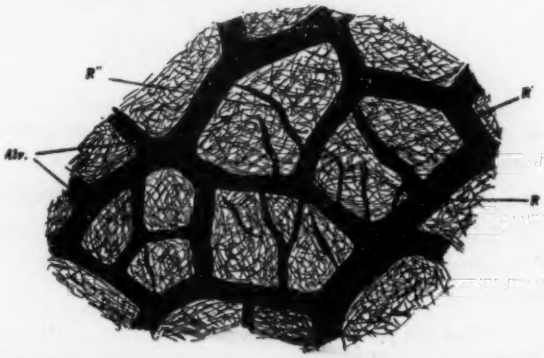
lungs is continuous and saccular, resembling that found in the Amphibia; and the respiratory surface is developed throughout, although rather less so in the posterior part. Whether these peculiarities are primitive or degenerate, is difficult to say. The glottis is a longitudinally directed, slit-like aperture,

situated on an elevation (*laryngeal prominence*) on the floor of the pharynx between the basal bifurcations of the tongue. It leads into the trachea. The latter runs ventral to the œsophagus and after traversing the cervical region, comes to lie dorsal to the heart where it befurcates into two extremely short *bronchi*. The anteriormost part of the trachea is dilated to form the larynx.



TEXT-FIG. 2. General dissection, showing the various viscera.—*c.*, cæcum; *d.*, duodenum. *g. b.*, gall-bladder; *ht.*, heart; *lg. lt.*, left lung; *lg. r.*, right lung; *lv.*, liver; *kd.*, kidney; *p.v.c.*, posterior vena cava; *Rct.*, rectum; *tr.*, trachea; *St.*, stomach.

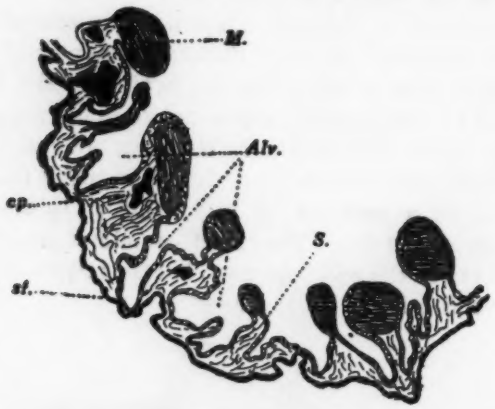
(*b*) *The Histology of the Lung.*—The lungs (Text-Fig. 2) are almost symmetrical structures, situated one on each side of the œsophagus, postero-lateral to the heart and lateral to the anterior half of the liver, which shows a contour particularly adapted to accommodate them. Each lung is a simple fusiform sac with thin transparent walls and complete septa inside. The wall of the posterior region of the lung is distinctly thinner than that of the anterior, and is not so richly supplied with blood capillaries. The cavity inside is continuous from one end to the other and there is no division into chambers, as found in many lizards. The inner lining is raised into a network of very delicate ridges, giving rise to a honeycomb-like appearance. The ridges are closer and more prominent in the anterior than in the posterior part, but the respiratory surface occurs throughout the entire lumen of the lungs.



TEXT-FIG. 3. Internal surface of the wall of a lung.—*Alv.*, alveoli; *R*, *R'*, *R"*, inter-alveolar ridges of different sizes; *sf.*, the superficial membrane.

The internal surface of each lung (Text-Fig. 3) shows a trellis-like network of ridges, which separate the *alveoli* from one another. The ridges are mainly of three sizes: one, fairly stout; the second, rather thick, though not so stout as those of the first type; and the third, extremely delicate, lying as slight elevations within the meshes formed by the ridges of the first two types. Each ridge is supported on a number of pillar-like strands, which are separated from each other by interspaces and thus allow the adjacent alveoli to be in communication. The ridges and their supporting strands are covered with more or less flattened endothelial cells, and serve to increase the respiratory surface of the lung. In a transverse section (Text-Fig. 4), the inter-alveolar ridges appear as relatively large knob-shaped structures situated at the top of delicate, more or less crumpled septa, projecting into the lumen of the lung. Each knob has a large muscle-band

inside, the shape of which differs according to the angle at which the section happens to pass through it. Muscular and fibro-elastic tissues are



TEXT-FIG. 4. Transverse section through a part of the lung.—*Alv.*, alveoli; *Cp.*, capillary; *M.*, muscle band at the distal end of the inter-alveolar ridge; *s.*, the supporting septum of the inter-alveolar ridge; *sf.*, the superficial membrane covering the lung.

also present within the supporting strands and numbers of blood capillaries can be made out, cut at various angles and interspersed in various parts of the section. The whole lung is covered by an extremely delicate superficial (serous) membrane.

(*c*) *The Mechanism of Respiration.*—Gnanamuthu (1933 and 1937) has given an excellent account of the mechanism of respiration in *Hemidactylus*. Not only does the 'thoracic' region expand and contract, but the posterior part of the throat also moves up and down, thereby diminishing and increasing the buccal space. As shown experimentally the buccal floor lowers when thorax dilates and rises when the chest contracts. These movements of the throat are not the passive effects of the inflation and deflation of the cavity by the entry and exit of air, but are active ones, due to the contraction and expansion of the buccal muscles.[1] According to Gnanamuthu (1937), the part played by the buccal floor in respiration is probably as follows:

"The contraction of the thorax expelling air would result in the inflation of the buccal cavity, and when next the thorax relaxes this impure air may be taken into the lungs again, because the thoracic contraction and expansion follow each other so rapidly. However, the elevation of the

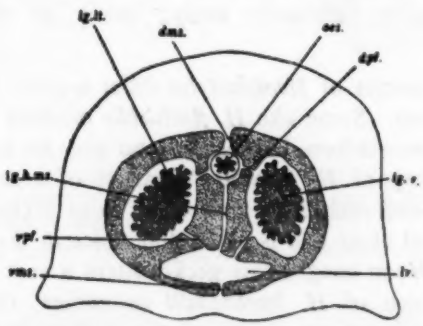
[1] As proved experimentally by Gnanamuthu (1937, pp. 47–48).

mouth-floor and tongue through the aid of the transverse and hyoid muscles just when the thorax contracts serves to expel the vitiated air effectively out of the body."

Whilst it cannot be denied that the buccal-floor movements may have some significance in preventing an accumulation of 'impure' air in the mouth cavity and consequently in preventing its entry again into the lungs, this sort of explanation is hardly adequate to account fully for the extremely delicate adjustments necessary for effecting an almost perfect synchronous working of the throat and the thoracic wall. Such a requirement could have been met merely by a greater development of rigidity in the buccal floor, rigidity just sufficient to cope with the extra air-pressure tending to be created in this region during expiration. What seems more likely, is, that the utility of the simultaneous movements of the throat and the thorax lies in making these two regions part of one harmonious respiratory mechanism, sucking in or forcing out air as a single structure. Exactly when the thoracic region expands, the buccal space gets also increased and the action of both in co-ordination sucks in air, as if a single chamber suddenly dilated to create a region of low air-pressure inside. Similarly their contractions synchronise to expel the foul air.

4. The Disposition of the Peritoneum in Relation to Lungs

Each lung (Text-Fig. 5) is suspended by two folds of the peritoneum: one, dorsal; and the other, ventral. The dorsal fold is attached to the



Text-fig. 5. Transverse section of the trunk, passing through the œsophagus and lungs. *dms.*, dorsal mesentery; *dpf.*, dorsal pulmonary fold; *g.h.ms.*, gastro-hepatic region of the mesentery; *oes.*, œsophagus; *vms.*, ventral (subhepatic) mesentery; *vpf.*, ventral pulmonary fold (Other abbreviations as in text-fig. 2).

lateral aspect of the œsophagus, and the ventral (which is situated towards the inner surface of the lung) the mesial mesentery connecting the

œsophagus with the liver. Thus on either side, a recess (*pulmo-hepatic recess*, Butler; *Pneumato-enteric recess*, Goodrich) is cut off from the general body cavity between the œsophagus, lung and liver, ending blindly in front, but opening behind into the cœlom.

As a transverse partition is absent, the 'thoracic' region is not morphologically separated from the 'abdominal'. However, physiologically, the same aim is achieved in a different way. The viscera are closely packed in the 'abdominal' region and thereby indirectly delimit the 'thoracic' region in front of them. On the right side, the liver is apposed to the body-wall, while on the left the stomach lies adpressed between the liver and the body-wall. Thus the space surrounding the lungs is fairly large, while in the region posterior to them it is virtually obliterated by the closely packed viscera. This arrangement subserves to restrict the effects of the expansion or contraction of cœlom to the part immediately surrounding the lungs. Were the space surrounding the lungs in communication with a large one behind, the effects would tend to distribute themselves over a wide area and be proportionately enfeebled. As far as I know, the importance of such a disposition for respiratory activity has never been mentioned before.

5. Voice

All geckos have a voice. As Smith (1935) says, "usually it is a soft chirruping or clucking sound such as we can make with our tongue, but some of the larger forms, such as *Gecko gecko*, have a loud cry that can be heard a considerable distance away; many of them squawk when captured."[2]

The various species of *Hemidactylus* differ a great deal in their power of sound production. Some like *H. flaviviridis* produce it only rarely, and then extremely low. Others like *H. frenatus* and *H. leschenaulti* cry fairly frequently. The cry of *H. frenatus* "consists of a series of six to nine guttural sounds—*chik-chik-chik*—uttered in close succession. The cry is often very loud and clear and is a familiar voice in the vicinity of wooden fences at dusk. When caught this gecko utters a sort of feeble squeaking sound."[3] The voice of *H. leschenaulti* resembles that of *H. frenatus*. However, "it sometimes utters at night a prolonged shrill sound which may be expressed as Shee-see-eek."[3] *H. garnoti* has also a rather loud voice and is called, on account of it, *Tjik Tjik* in the Malayan language.[4]

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[2] Smith, M. A., "Reptilia and Amphibia, Vol. II, Sauria," *Fauna Brit. Ind.*, 1935, p. 28.

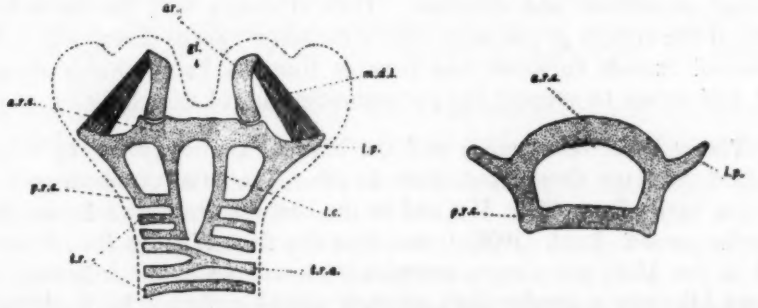
[3] Y. R. Rao in *St. Joseph College (Trichinopoly) Magazine*. Sept. 1915.

[4] Volz, W., "Lacertilia von Palembang," *Zool. Jahrb.*, 1903, 19.

6. The Skeleton of the Larynx, Trachea and Bronchi

The skeleton of the air-passage was studied in seven individuals by means of van Wijhe's process of cartilage staining. As previous authors (Henle, 1839; Steck, 1908) did not have recourse to such a delicate method, the author is in a position to give a more accurate description than has been hitherto possible, as well as to add notably to our knowledge of the subject.

The wall of the *larynx* is supported by a ring-shaped *cricoid* projecting laterally to form obtuse processes and a pair of *arytenoids* fused to the antero-dorsal aspect of the cricoid on either side and bounding the glottis on the right and left sides. Gengenbaur and others held that the cricoid, arytenoids and perhaps the tracheal rings are derived from the fifth branchial arch;—a view which Goodrich (1930, p. 446) criticized on the ground that the evidence is incomplete. According to Henle (1839), the larynx arises from two lateral cartilages which send transverse processes to meet each other in front of and behind the air-passage.



Text-fig. 6. Dorsal view of the laryngeal skeleton.—*ar.*, arytenoid; *a.r.c.*, anterior ring of the cricoid; *gl.*, glottis; *l.c.*, longitudinal connecting cartilages; *l.p.*, lateral process of the arytenoid; *m.d.l.*, musculus dilatator laryngis; *p.r.c.*, posterior ring of the cricoid; *t.r.*, tracheal rings; *t.r.a.*, abnormal tracheal ring.

Text-fig. 7. Ventral view of the laygneal skeleton.—Abbreviations as in the previous figure.

The *cricoid* of *Hemidactylus flaviviridis* (Text-Figs. 6-7), although composed of two successive rings as that of *Hemidactylys garnoti* (Steck, 1908) differs from the latter in many respects:

In the first place, the anterior ring in *Hemidactylus garnoti*, according to Steck (1908), is open dorsally, while the posterior ring is closed completely all round. In *Hemidactylus flaviviridis*, the anterior ring is closed dorsally as well as ventrally; while the posterior ring is open dorsally.

Secondly, the anterior ring in *Hemidactylus garnoti* is produced mesially at its antero-ventral aspect into a pointed edge which was erroneously called the processus epiglotticus, but may be more accurately designated as *Processus anterior inferior* (Göppert). In *Hemidactylus flaviviridis* the antero-ventral border of this ring is rounded and the processus anterior inferior is altogether absent.

Thirdly, there is no median projection in *H. flaviviridis* corresponding to the *processus anterior superior* of *H. garnoti*.

Fourthly, in *Hemidactylus flaviviridis* a pair of longitudinal cartilages join the dorsal part of the first ring with the dorsolateral portions of the second ring. These cartilages extend even farther back to join the first two or three tracheal rings. They are absent in *H. garnoti*; in which, however, the second, third and fourth tracheal rings are often irregularly interconnected with each other (Steck, 1908).

The arytenoids of *Hemidactylus flaviviridis* resemble those of *H. garnoti* in their attachment and direction. They articulate with the dorso-lateral parts of the cricoid at the place where the latter sweeps downwards. Each arytenoid extends forwards and inwards from its base towards its distal end, and serves to support the corresponding lip of the glottis.

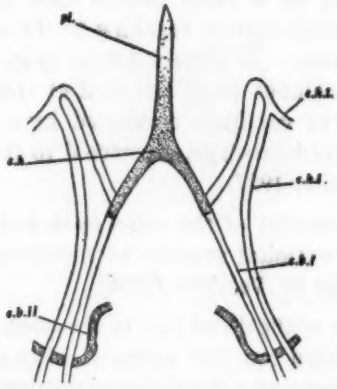
The walls of the trachea and the branchi are supported by rings of calcified cartilage throughout their length. The number of rings in the trachea varies from 45 to 51; and in the bronchi from 6 to 8. In *Hemidactylus garnoti*, Steck (1908) found that the first 27 rings (*i.e.*, from the 2nd to the 28th) are always completely closed, while the following ones (about 14) show a slender cleft on their dorsal surface. In *H. flaviviridis* most of the rings are closed, there being extremely few incomplete rings. Some of the rings are abnormal, as they are divided asymmetrically into anterior and posterior half-hoops.

The bronchial rings, usually 6 to 8 in number, are incomplete towards their inner sides. They are variously connected to each other to form an irregular framework, supporting the walls of the bronchi. The last ring is always the largest.

Tiedemann (1818) and Meckel (1818) described a dilatation of the trachea in *Ptyodactylus fimbriatus* and regarded it as a supplementary voice-producing apparatus. In *Hemidactylus flaviviridis*, as well as in *H. garnoti* (Steck, 1908), there is no trace of such a structure.

7. The Hyoid Apparatus

The hyoid apparatus (Text-Fig. 8) consists of a central portion, the *basihyoid* (*corpus hyale*), a median anterior process, the *processus lingualis* (*entoglossus*), two pairs of cornua attached to the basihyoid, the *cornua*



Text-fig. 8. The Hyoid Apparatus.—*c.b.I* and *c.b.II*, first and second ceratobranchials; *c.h.*, corpus hyale; *c.h.l.* and *c.h.l'*, the two limbs of the corner hyale; *p.l.*, processus lingualis.

hyale and the *cornua branchialia I* (*first ceratobranchials*), and the vestiges of a third pair of cornua, the *cornua branchialia II* (*second ceratobranchials*).

The *basihyoid* is a triradiate structure lying mesially below the trachea a little behind the larynx. Its anterior median arm is continued into the base of the tongue as a slender process, the *processus lingualis* or *entoglossus*, whilst its posterior two arms, rather stouter, are movably articulated to the pair of *first ceratobranchials*. The *cornu hyale* of each side is attached to the basihyoid just anterior to the articulation of the latter with the first ceratobranchial. It extends forwards and outwards in a broad curve with the concavity facing backwards. Distally, it is divided into two processes: a short and straight lateral limb directed obliquely outwards, and a delicate long limb, at first projecting longitudinally backwards and then curving dorsalwards to end underneath the paroccipital process.

The *cornu branchiale I* of each side is a well developed process, ossified throughout its extent. It curves upwards in its distal portion and ends, like the corresponding region of the cornu hyale, at the parotic region of the skull.

Gnanamuthu (1937) regarded the *cornua branchialia II* as absent in *Hemidactylus* and certain other lizards, but alizarin preparations show that

they are present as extremely slender rod-like vestiges near the distal end of the *cornua branchialia I.* Their proximal portions, however, are lacking.

As described above, the hyoid apparatus of *Hemidactylus flaviviridis* Rüppel is characterized by the following new features:

1. The occurrence of a blunt lateral limb on the *cornu hyale* in addition to the usual prolongation reaching to the parotic region has been recorded for the first time. In certain lizards (e.g., *Mabuya, Cabrita,* etc.) there is a small cartilaginous plate attached at this place, and in *Calotes* there is a short rod-like cartilage developed here. In *Varanus,* there is a peculiar crotchet-shaped cartilage, connected to the proximal part of the *cornu hyale* (Gnanamuthu, 1937).

2. The distal extremities of the *cornu hyale* and the *cornu branchiale I* end ventral to the paroccipital process as mentioned for *Gekkonidæ, Uroplatidæ* and *Eublepharidæ* by Versluys (1936).

3. The occurrence of the distal part of the *cornu branchiale II* is remarkable. As Versluys pointed out, this cornu is often divided into two parts, a proximal ventral portion and a distal dorsal portion. The former is absent and the latter present in *Hemidactylus flaviviridis.*

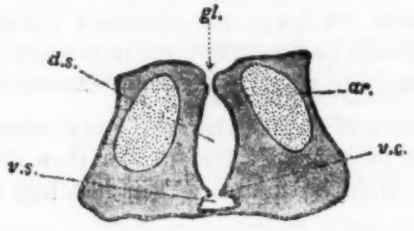
8. The Soft Parts of the Larynx

As in other lizards, the larynx possesses two pairs of muscles: (1) *M. compressor laryngis,* arising from the body of the hyoid and inserted on the borders of the cricoid and arytenoids and (2) *M. dilatator laryngis* arising from the lateral processes of the cricoid and inserted on the arytenoids. The former serves as a sphincter and the latter as the dilatator of the glottis.

The laryngeal nuscles are supplied by two branches of the vagus nerve: an anterior one, called the *Nervus laryngeus superior,* which corresponds to the first branchial branch of Fishes; and a posterior one, called the *Nervus laryngeus inferior* or *recurrens,* which represents the fourth branchial nerve and belongs to the seventh visceral arch in Fishes.

In *Hemidactylus garnoti,* according to Steck (1908), there is neither a local dilatation of the trachea as in *Ptyodactylus fimbriatus,* nor a specially wide trachea as in *Platydactylus guttatus.* There are also no vocal cords as described by other authors in the Gekkonidæ. In *Hemidactylus flaviviridis,* however, the vocal cords are definitely present. They are not in the form of elastic bands stretched between the dorsal and ventral walls of the cricoid, as described for Gekkonidæ by previous authors (Henle, 1939, etc.), but are prominent horizontal folds of the epithelial lining of the larynx

(Text-Fig. 9). They lie in the anteriormost part of this chamber on the lower part of the lateral walls; and when fully extended, virtually divide an extensive dorsal space from an extremely small ventral one. The former space is supported by the arytenoids and opens at the glottis. The



TEXT-FIG. 9. Transverse section through the anterior part of the larynx, showing the vocal cords.—*d.s.*, the dorsal laryngeal space in communication with the glottis; *v.c.*, vocal cords; *v.s.*, ventral laryngeal space leading posteriorly into the trachea.

latter, when traced backwards in serial transverse sections, is seen to lead into the trachea.

9. THE MECHANISM FOR THE PRODUCTION OF VOICE

According to Steck (1908), the absence of tracheal dilatations and vocal cords in *Hemidactylus garnoti* is correlated with a number of anatomical peculiarities. The bases of arytenoids are shifted towards the dorsal side, so that the Ligamenta aryhoidea are more strongly developed. The dorsal borders of the arytenoids take no part in the formation of the glottis. The mucous membrane, which would in other cases cover both these cartilages as simple ridges called *Plicæ arytænoideæ*, is developed as a ligamentum arycricoideum. The glottis is abnormally long, since the first laryngeal ring is incomplete dorsally and the ligamentum arycricoideum extends up to the second laryngeal ring. The voice, accordingly, is produced by the vibration of the dense arycricoid ligament when it is tensely stretched and is enhanced by the floor of the mouth, which possesses a transversely striped musculature.

In *Hemidactylus flaviviridis*, however, the mechanism is different. Here, as shown by me above, the vocal cords are well developed. When fully extended, they partially separate a dorsal chamber from a ventral one. The stream of air expelled forcibly from the lungs, passes up from the ventral chamber into the dorsal one and gets out at the glottis. This sets the vocal cords into vibration and produces the sound.

10. Summary

The author has described the respiratory and vocal organs of *Hemidactylus flaviviridis* in detail, the more important features discovered by him being as follows:—

1. The right and left lungs are equal and symmetrical, with their internal cavities undivided and saccular; the respiratory surface is developed throughout; and there are no intrapulmonary extensions of the bronchi.

2. The inner lining of the lungs is raised into a network of ridges, which are closer and more prominent in the anterior than in the posterior part. The ridges are mainly of three sizes. Their histology has been described in detail.

3. The significance of the simultaneous movements of the throat and thorax has been pointed out.

4. The disposition of the peritoneum in relation to lungs has been studied, and the rôle of the viscera in restricting the effects of the expansion or contraction of the cœlom to the part surrounding the lungs has been pointed out.

5. The *cricoid* of *Hemidactylus flaviviridis* differs from that of *H. garnoti* in the structure of its component rings, in the absence of the processus anterior inferior and anterior superior, and in the presence of a pair of dorsal longitudinal connecting cartilages.

6. The number of tracheal rings varies from 45 to 51 and of bronchial ones from 6 to 8. Most of the former are closed. The latter are incomplete towards their inner sides.

7. There is no dilatation of the trachea.

8. The hyoid apparatus is characterized by the development of a blunt lateral limb on the *cornu hyale*, by the ending of the *cornu hyale* and *cornu branchiale I* ventral to the paroccipital process, and by the presence of a vestigial *cornu branchiale II*.

9. The larynx is provided with two pairs of muscles (*M. compressor laryngis* and *M. dilatator laryngis*), which are innervated by the *Nervus laryngeus superior* and the *N. laryngeus inferior*.

10. The vocal cords are present. When fully extended, they virtually separate a dorsal laryngeal chamber from a ventral one.

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ON THREE COCCIDIAN PARASITES *WENYONELLA MACKINNONI* N.SP., *EIMERIA LUCKNOWENSIS* N.SP., AND *ISOSPORA* SP., FROM THE INTESTINE OF THE WAGTAIL *MOTACILLA ALBA* LINN. (PASSERIFORMES, MOTACILLIDÆ)

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INTRODUCTION

DURING the winter of 1940, eight specimens of the common wagtail *Motacilla alba* Linn. were entrapped in Lucknow and an examination of their droppings revealed a coccidial infection in two out of eight birds. In order to study the exogenous stages of development of this coccidian, the droppings as well as the rectal contents of the infected birds after dissection were kept in 1 per cent. solution of chromic acid. Each oocyst, after sporulation, showed four sporocysts inside it, and each sporocyst in turn had four sporozoites, *i.e.*, four sporocysts and sixteen sporozoites were present in each oocyst—a diagnostic character of the genus *Wenyonella* Hoare, 1933.

During the winter of 1941, six more specimens of the same bird were examined for coccidial infection; out of these five proved to be coccidia-free, but one was passing two kinds of oocysts along with its fæces: (i) oval oocysts, which were colourless, and (ii) spherical oocysts with thick yellowish inner cyst walls. In 1 per cent. solution of chromic acid, after complete sporulation, these oocysts were diagnosed as belonging to the genera *Eimeria* Schneider, 1875, and *Isospora* Schneider, 1881, respectively.

The coccidia-free specimens of *Motacilla alba* could not be infected artificially, as they died, (probably they could not stand confinement for long), before the oocysts of the three above-mentioned coccidian parasites could sporulate and attain the infective stage in the culture medium.

Pieces of small intestine were fixed in Bouin-Duboscq-Brazil, sectioned 4–6 μ thick, and stained with iron-alum hæmatoxylin and chromotrop 2 R, or Delafield's hæmatoxylin only. A few fresh smears of the scrapings of the intestine were made in normal saline solution and examined under an oil-immersion lens, but no motile stages of the parasites could be detected. Similar smears fixed in Schaudinn's fluid and stained with iron-hæmatoxylin were also examined but they did not yield any significant result besides those that had been obtained from a study of the sections of the intestine.

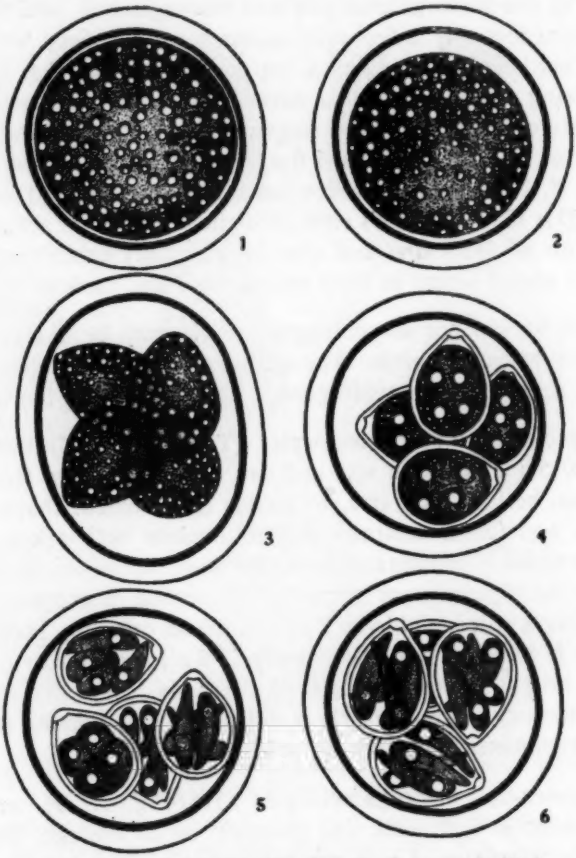
It may be mentioned here that only six species of *Wenyonella* have been recorded up to date (*vide* Table I). However, the species of *Wenyonella* described in this paper differs from those mentioned above in certain particulars, and therefore, I propose to designate this coccidian of the wagtail as *Wenyonella mackinnoni* n.sp., the specific name being given in honour of Prof. Doris L. Mackinnon of the King's College, London.

Wenyonella mackinnoni n.sp.

Exogenous stages.—The oocysts are spherical or ovoid in shape; they measure 19 μ–23 μ in spherical forms, and 23·8 μ–26·2 μ × 18·0 μ–21·5 μ in ovoid forms. The cyst wall consists of two layers: an outer layer which is thin and colourless, and an inner layer which is comparatively thicker and is brownish in colour. The protoplasm of the freshly discharged oocyst is filled with refractile granules of reserve materials and occupies the entire internal space (Text-Fig. I, 1), but later on it becomes condensed and has a more or less spherical contour and measures, on an average, 15·5 μ, in diameter (Text-Fig. I, 2); the micropyle and the polar inclusions are absent in the oocysts.

Oocysts kept in 1 per cent. solution of chromic acid and examined at regular intervals of six hours at room temperature revealed visible signs of segmentation of the zygotes within 24 to 36 hours, and within next 48 to 60 hours four rounded bodies, the sporozoites, were cleaved out of the protoplasmic bulk of the zygote. Usually the sporoblasts remain adhering together for some time, but later on they separate (Text-Fig. I, 3), become ovoid, and each of them secretes a wall around itself and thus forms the sporocyst (Text-Fig. I, 4). There is no oocystic residue left after the formation of the sporocysts. Each sporocyst measures 10·2 μ × 7·4 μ in

size, and has a lens-shaped thickening at its narrower end. The protoplasm of the sporocyst in turn segments, without leaving any residue, into four rounded bodies, the precursors of the sporozoites, which later on elongate (8·2 μ long) and assume a club-shaped appearance (Text-Fig. I



(All figures were drawn with the aid of camera lucida.)

TEXT-FIG. I.—Showing exogenous stages of *Wenyonella mackinnoni* n. sp. From living specimens × 1900.

1, a freshly discharged oocyst. 2, oocyst with unsegmented but condensed zygote. 3, oocyst showing formation of sporoblasts. 4, oocyst with four sporocysts. 5, 6, oocysts showing sprocysts, each wirh four sporozoites.

5, 6). They are arranged at random inside the sporocysts. The formation of the sporozoites takes place during the next 24 to 48 hours after the formation of the sporocysts, *i.e.*, complete sporulation takes 4 to 6 days.

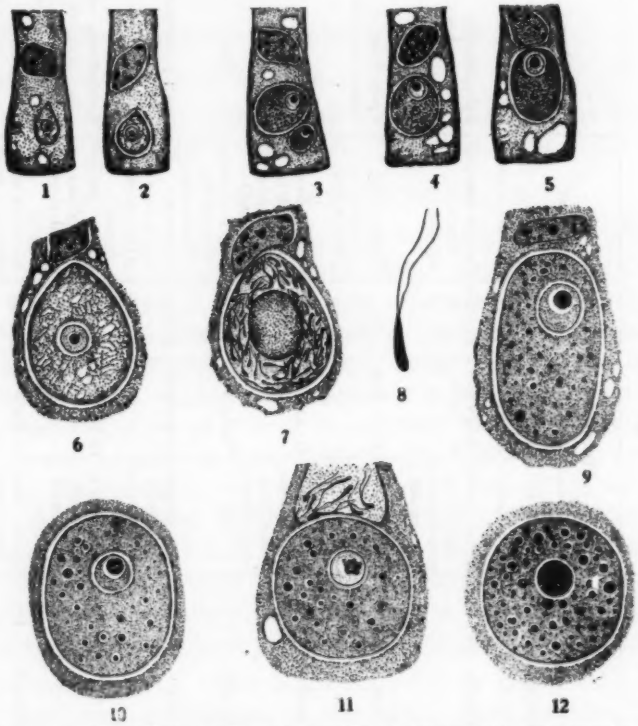
Endogenous stages.—The endogenous cycle of development takes place in the small intestine of the host. It may be mentioned at once that no asexual or schizogonic stages of the parasite could be detected, and the only stages frequently encountered were: (i) the microgametocytes and microgametes, (ii) the macrogametocytes and macrogametes, and (iii) the zygotes. On one occasion only a few microgametes were noticed within the cavity inside an epithelial cell lodging a macrogamete (Text-Fig. II, 11); but no stage showing the entrance of a microgamete into the macrogamete could be encountered. Besides these stages, young developmental stages of the sexual forms, measuring $2{\cdot}2\,\mu$–$6{\cdot}0\,\mu$, were also seen on certain occasions (Text-Fig. II, 1–5): they have been designated as the sexual forms, following Ray and Das Gupta's (1937) view of distinguishing the schizonts from the sexual forms of *W. hoarei* and also because they exhibit resemblances to the mature sexual forms in their cytoplasmic and nuclear contents.

The entire absence of schizogonic cycle may be due to the fact that it was over when the birds were examined. It seems that this parasite, like other coccidia, also undergoes a course of "self-limited" infection.

The grown-up microgametocytes (Text-Fig. II, 6) measure, on an average, $20{\cdot}5\,\mu \times 15{\cdot}6\,\mu$ in size and can be distinguished from the macrogametocytes, besides their size, by having (i) a conical shape, (ii) a ragged cytoplasm, and (iii) a centrally located nucleus with a centrally situated karyosome which is comparatively smaller than that of the macrogametocyte. A mature microgametocyte gives rise to several microgametes (Text-Fig. II, 7), leaving a considerable bulk of cytoplasm unused. Each microgamete (Text-Fig. II, 8) has an elongated body ($3{\cdot}8\,\mu$ long) and two equal flagella which are nearly twice the length of the body. Whether the flagella are attached anteriorly or posteriorly is difficult to say, because the movements of the microgametes could not be observed *in vivo*.

The grown-up macrogametocytes (Text-Fig. II, 9) are ellipsoidal bodies, rounded at both ends, and measure, on an average, $28{\cdot}5\,\mu \times 16{\cdot}0\,\mu$ in size. The cytoplasm of each macrogametocyte contains reserve materials, and the nucleus lies rather nearer the superior pole. The karyosome is fairly big and excentric in position, being surrounded by a clear space. During the course of its development the macrogametocyte becomes more or less globular in shape, and gives rise to a single macrogamete (Text-Fig. II, 10, 11) measuring $23{\cdot}0\,\mu \times 19{\cdot}8\,\mu$ in size. On no occasion could a micropyle be detected in the macrogametes of *Wenyonella mackinnoni* (cf. *W. hoarei* Ray and Das Gupta, which posseses a prominent micropyle.

The zygotes (Text-Fig. II, 12) can be distinguished from the macrogametocytes and macrogametes by having (i) a denser accumulation of reserve materials (the so-called plastic and hæmatoxylinophilic granules), and (ii) a homogeneously stained nucleus.



TEXT-FIG. II.—Showing endogenous stages of *Wenyonella mackinnoni* n. sp. From sections of small intestine.

Figs. 1–5. Developing sexual forms × 1750. 1, 2, microgametocytes. 3, 4, 5, macrogametocytes; in fig. 3 two parasites are seen in a single cell. Figs. 6–12. Mature sexual forms. 6, a microgametocyte × 1000. 7, showing several microgametes and a central "restkörper" (semi-diagrammatic) × 1000. 8, a highly magnified microgamete × 2500. 9, a macrogametocyte × 1050. 10, 11, macrogametes; in fig. 11 a few microgametes are seen lying near the macrogamete × 1050. 12, a zygote × 1050.

Diagnosis.—Tetrazoic tetrasporocystid condition of the oocysts determines the position of this coccidian under the genus *Wenyonella* Hoare, 1933.

Oocysts spherical or ovoid, measuring 19·0 μ–26·2 μ × 18·0 μ–21·5 μ; cyst wall thick, double-layered, outer colourless, inner brownish; micropyle absent; sporocysts ovoid, measuring 10·2 μ × 7·4 μ, with a lens-shaped

TABLE I

Comparison between the different species of Wenyonella.
The measurements are given in microns.

Name	Oocysts			Sporulation period	Sporocysts			Host	Habitat	Locality
	Shape	Measurements	Residue		Shape	Measurements	Residue			
1 *W. africana*, Hoare, 1933	Subspherical or ovoid	18·5—19·2× 16·0—17·6	Absent	5–7 days	Ovoid, lensiform knob present at the narrower pole	9·6×8·0	Present	*Boaedon lineatus* (Ophidia, Reptilia)	Subepithelial tissues of small intestine	Entebbe, Africa
2 *W. hoarei* Ray and Das Gupta, 1935	Spherical	14·9—18·5	Do	7 ,,	Do	10·0×8·0	Do	*Sciurus* sp. (Rodentia, Mammalia)	Epithelium of small intestine	Calcutta, India
3 *W. uelensis* Berghe, 1938	Ovoid	26·0—30·0× 19·0—20·0	Transient	5 ,,	Ovoid, both ends similar, lensiform knob absent	11·4×7·6	Insignificant	*Funisciurus anerythrus* (Rodentia, Mammalia)	Probably the intestine	Belgian Congo, Africa
4 *W. parva* Berghe, 1938	Subspherical	15·2—13·3	Absent	7 ,,	Do	7·6×5·0	Do(?)	*Tamiscus emini* (Rodentia, Mammalia)	Do	Do
5 *W. bakli* Misra, 1944	Subspherical or ovoid	16·0—17·5× 14·6—15·5	Do	4–5 ,,	Egg-shaped lensiform knob absent	6·6×4·2	Absent	*Coturnix communis* (Galliformes, Aves)	Small intestine	Lucknow India
6 *W. gallinae*, Ray, 1945	Oval or egg-shaped	29·48-33·50× 19·84-22·78	?	4–6 ,,	Short necked roundbottomed vials	18·76×8·04	Present	*Gallus gallus domesticus* (Galliformes, Aves)	Epithelium of the terminal part of the intestine	Mukteswar, Kumaun, India
7 *W. mackinnoni* n. sp.	Spherical or ovoid	19·0—26·2× 18·0—21·5	Absent	Do	Ovoid, lensiform knob present at narrower pole	10·2×7·4	Absent	*Motacilla alba* (Passeriformes, Aves)	Epithelium of small intestine	Lucknow, India

knob at one end; sporozoites 8·2 μ long, club-shaped, irregularly arranged; oocystic and sporocystic residual bodies absent; sporulation time 4 to 6 days; unsegmented oocysts discharged in the fæces of the host.

Systematic position.—***Wenyonella mackinnoni*** n.sp. (Eimeriidæ, Coccidiida).

Habitat.—Small intestine of ***Motacilla alba*** Linn.

Locality.—Lucknow, U.P., India.

The accompanying table shows a comparison of the known species of ***Wenyonella*** with regard to oocysts, sporocysts, hosts, etc.

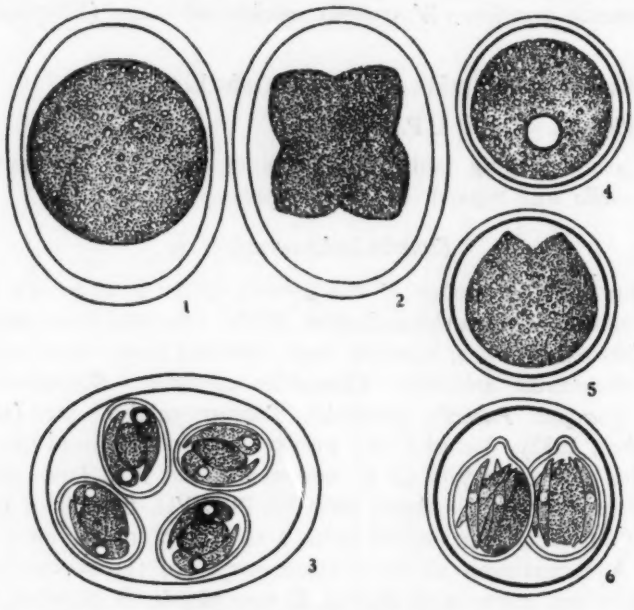
Eimeria lucknowensis n.sp.

To the best knowledge of the author, there is only one species of ***Eimeria***, namely, ***E. roscoviensis*** (Labbé, 1893)* recorded from ***Motacilla alba*** Linn. This parasite has, however, been reported from other birds as well, e.g., ***Phalacrocorax aristotelis, Charadrius cantianus, Strepsilas interpres, Numnius phæopus, Pulvialis apricarius, Totanus calidris,*** etc. (***vide*** Levine and Becker, 1933). Labbé in his previous paper (1893) did not give any illustration of the oocysts of ***E. roscoviensis***, but in a later contribution (1896) he supplemented a figure (***vide*** his Pl. XVII, fig. 18) of the mature oocyst of this coccidian, which gives a clear idea of the structure of its oocyst. A comparison of the mature oocyst of the species of ***Eimeria*** described in this paper with that of ***E. roscoviensis***, as sketched by Labbé, would at once reveal that the latter species does not coincide in its characters with the former, and therefore, a new specific name, ***Eimeria lucknowensis*** n.sp., has been instituted for the present coccidian.

The oocysts of ***E. lucknowensis*** are ovoid in shape and are eliminated in an unsegmented condition in the fæces; they measure 21·4 μ–24·5 μ × 17·4 μ–18·8 μ in size. The cyst wall is colourless and double-layered; both ends of the oocysts are similar and rounded, and there is no indication of a flattening or prolongation at either end, nor is there any evidence of a micropyle and polar inclusions. In these characters ***E. lucknowensis*** differs markedly from ***E. roscoviensis***, in which the oocysts are pyriform in shape, measure 16·0 μ–18·0 μ × 14·0 μ–16·0 μ, and each mature oocyst is characterized by the presence of a truncated neck bearing a pseudomicropyle, as well as the polar globules. In 1 per cent. solution of chromic acid the oocysts of ***E. lucknowensis*** sporulate within 3 to 4 days. There is no residual body inside the oocyst after the formation of the sporoblasts,

* Labbé in his original paper (1893, p. 408) has named it as *Coccidium roscoviense*.

which are four in number, and which later on secrete a wall around each one of them, thus giving rise to the same number of sporocysts (Text-Fig. III, 1–3). Each sporocyst is ovoid in shape, and is devoid of any thickening



TEXT-FIG. III.—Showing sporulation in *Eimeria lucknowensis* n. sp. and *Isospora* sp. From living specimens × 1750.

1, oocyst of *E. lucknowensis* n. sp., with unsegmented zygote. 2, showing formation of sporoblasts. 3, an infective oocyst with four sporocysts, each with two sporozoites and a residual body. 4, a freshly voided oocyst of *Isospora* sp. showing formation of two sporoblasts. 6, an infective oocyst with two sporocysts, each containing four sporozotsei and a voluminous residual body.

at either pole; it measures $8{\cdot}5\mu \times 6{\cdot}0\mu$. The end-product of sporogony is the formation of two club-shaped, curved sporozoites within each sporocyst; the sporozoites measure $7{\cdot}0\mu$ in length and are arranged with their concavities facing the sporocystic residuum between them. The sporocyst of *E. roscoviensis*, on the other hand, is pyriform in shape, and has a knob-like thickening at its narrower pole; moreover, the two sporozoites in each sporocyst lie on one side, the other side of the sporocyst being occupied by the residual body.

A study of the endogenous stages found in the small intestine was not conclusive, because of the simultaneous presence of the endogenous stages of another coccidium *Isospora* sp. described below.

Diagnosis.—Dizoic tetrasporocystid condition of the oocysts places this coccidian under the genus *Eimeria* Schneider, 1875.

Oocysts ovoid, 21·4 μ–24·5 μ × 17·4 μ–18·8 μ, discharged unsegmented in the fæces; sporocysts ovoid, 8·5 μ × 6·0 μ; sporocystic residue in between the two sporozoites; sporulation period 3 to 4 days.

Systematic position.—*Eimeria lucknowensis* n.sp. (Eimeriidæ, Coccidiida).

Habitat.—Small intestine of *Motacilla alba* Linn.

Locality.—Lucknow, U.P., India.

Isospora sp.

Only one coccidian belonging to the genus *Isospora*, namely, *I. passerum** Sjöbring, 1897, has been reported from *Motacilla alba* Linn. This parasite, however, has been recognised as a synonym of *Isospora lacazei* (= *Diplospora lacazii*) Labbé, 1893, of the passerine birds, and held by certain workers to be a pathogenic species. Thus Labbé (1893) mentioned that *I. lacazei* proved fatal to finches infected experimentally with sufficient doses of this parasite. Hadley (1910) asserted that the common English sparrow and other birds, if chanced to find access into the poultry runs, could transmit† white diarrhœa to young fowls and blackhead to turkeys, the causative agent being the same parasite. Becker (1934) stated that this "parasite has a special interest because it is a cause of loss among caged birds, particularly canaries".

Existence of more than one species of *Isospora* in passerine birds has been suggested by several protozoologists, *e.g.*, Labbé (1893), Wenyon (1926), Becker (1934), etc., but cross-infection experiments have not been conducted to support their views. Labbé (1893), however, recorded *Isospora rivoltæ* (= *Diplospora rivoltæ*) from chaffinch, speckled magpie and titmouse (all passerines), the distinguishing characters of this coccidian being the comparatively heavier wall of its oocysts, and the oocysts required not less than 15 days (Labbé in 1896 mentions "douze a quinze jours") for development, whereas in *I. lacazei* the walls of the oocysts are thinner, and the oocysts required 4 to 5 days (Labbé in 1896 mentions "trois ou quatre jours") for sporulation. Although Labbé has given no illustrations of *I. rivoltæ*, the above-mentioned differences, as well as the differences in the measurements of the oocysts (in *I. lacazei* 23 μ–25 μ and in *I. rivoltæ* 16 μ–18 μ), are,

* Also known as *Isospora communis-passerum* Sjöbring, 1897.

† Hadley's announcement of the pathogenicity of this parasite has, from cross-infection experiments, been proved untenable by Smith and Smillie (1917), Johnson (1923), Boughton (1929), etc., since all attempts to infect fowls with this coccidian have met with failure.

I think, quite suggestive of regarding these two parasites as distinct and separate species.* Becker (1934, p. 101) has also expressed that the species of *I. rivoltæ*, "as well as some new ones, may have to be recognised". However, the present species of *Isospora* differs in certain respects from *I. lacazei*, but in the size of its oocysts, comparatively thicker cyst walls, and delayed period of sporulation, it approximates to *I. rivoltæ*, and therefore, it has been avoided to dub a new specific name to it. The distinguishing characters of this coccidian are given below.

The oocysts are spherical, 14·8 μ–17·8 μ in diameter, and are discharged in an unsegmented condition along with the fæces of the host; the cyst-wall is two-layered, the inner layer being comparatively thick and yellowish in colour, while the outer one is thin and colourless; micropyle and polar inclusions are absent; sporulation (Text-Fig. III, 4–6) in 1 per cent. solution of chromic acid requires 10 to 12 days; two sporocysts are formed in each oocyst and the oocystic residue is absent. Each sporocyst, measuring 10·6 $\mu \times$ 7·4 μ, is ovoid in shape having one pole rounded and the other narrower, the latter having a nipple-like knob at its extremity; the Steida body is invariably absent in the sporocyst. The contents of the sporocysts undergo segmentation and thus four spindle-shaped sporozoites measuring 7·5 μ in length are formed, and a voluminous residue is left inside each sporocyst. The arrangement of the sporozoites does not follow any regular order.

Endogenous stages were not conclusive due to a mixed infection (*vide supra*).

Diagnosis.—Tetrazoic disporocystid condition of the oocysts locates this coccidian under the genus *Isospora* Schneider, 1881.

Oocysts spherical, 14·8 μ–17·8 μ, unsegmented in fresh fæces; sporocysts ovoid, with nipple-like knob, 10·6 $\mu \times$ 7·4 μ; sporulation time 10 to 12 days.

Systematic position.—*Isospora* sp. (Eimeriidæ, Coccidiida).

Habitat.—Small intestine of *Motacilla alba* Linn.

Locality.—Lucknow, U.P., India.

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* The coccidian *I. rivolta* Grassi, 1879, which inhabits the intestines of cats and dogs, has been mentioned as *I. rivoltæ* by certain writers, *e.g.*, Leuckart (1886, p. 221, *Coccidium rivoltæ*), Dobell and O' Connor (1921, p. 98), etc. If *I. rivoltæ* (Labbé, 1893) is recognised as a valid species, it is suggested, in order to avoid confusion between the two different parasites —the one occurring in cats and dogs and the other in birds, that the name *I. rivoltæ* should be substituted by *I. labbei*, n. n.

ACKNOWLEDGMENTS

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STUDIES ON THE REFRACTIVE INDEX OF MILK

Part I. Observations on Genuine Samples

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(Communicated by Mr. M. Sreenivasaya, B.A., F.I.I.SC , F.A.SC.)

Various official tests, physical and chemical, have been devised for the determination of added water in milk. The principal ones are the well-known presumptive standards for fat and solids-not-fat of milk, the cryoscopic test (Beckmann, 1894) and the refractive index of the sour (Leach and Lythgoe, 1903), acetic (Leach and Lythgoe, 1904) and copper sulphate serum (1910) of milk, prepared under standard conditions. The preparation of the milk-serum has been resorted to owing to the opacity of milk when viewed through the immersion refractometer. The time-consuming chemical procedure of this method has brought about the popularity of the cryoscopic test, although the latter needs a considerable amount of skilled technique in its measurement.

In this paper standardisation of a simple and quick method of determining the refractive index of milk, in contradistinction to that of milk-serum, with the Abbé Refractometer has been attempted. The cow and buffalo being equally common milch animals in India, the range of variation of R.I. for each type of milk has been studied. Further, the relationship between the density and the R.I. (*i.e.*, Refractive constant, K) have also been calculated for a large number of samples. The data from all these determinations have been statistically analysed.

Experimental

The R.I. determinations with the Abbé Refractometer were made, for the first few samples of milk, on whole milk. But it was found that the presence of fat in milk necessitated a very quick adjustment of the total line of reflection in the refractometer, as otherwise the line tended to blur and flow with delay. Defatted milk, therefore, gave a sharper and more permanent line of demarcation without affecting the measure of the R.I., thus permitting a greater degree of accuracy and freedom in the determination. Table I illustrates this fact.

TABLE I

R.I. (40° C.) *of whole and skimmed milk*

Cow		Buffalo	
Whole	Skimmed	Whole	Skimmed
1·3450	1·3451	1·3470	1·3472
65	66	68	86
52	51	61	60
65	64	77	76

After a few trials the following method was finally adopted. 10 c.c. of sample is pipetted into a Gerber butyrometer and centrifuged for 5 minutes when almost all the fat forms a plug on top leaving skimmed milk at bottom. A few c.c. of the latter is carefully collected in a test-tube without disturbing the disposition of the two layers, and the R.I. determined on the skim milk. The readings were taken when the temperature of the instrument was steady at 40° C., and repeated with fresh drops until the difference between consecutive readings did not exceed 0·0003.

Samples of milk for examination were mostly obtained from the Military Dairy Farm, Hebbal, about 3 miles from the Institute. About 1 to 2·5 hours lapsed between milking and the analysis of the samples, the time lapse causing no detectable difference in the R.I.

The farm has about 400 milking animals, the cows belonging to Scindhi, Tharparkar, Ongole, Cross (Ayrshire × Indian) and C.P. breeds, and the buffaloes to Delhi, Nagpur and Neeli breeds. Samples were collected both from individual animals as well as pooled milk, both chosen, as far as possible, at random from the herd. The bulk samples were collected from cans containing the yield of 15–25 animals. About half the number of cows were suckled by calves before milking, but the buffaloes were all milked without this practice. The animals were in all stages of lactation from 15 days to about 8 months after parturition. Samples were collected in the morning (9 to 11 in summer and 7 to 8 in the rainy season) by the laboratory attendant, in whose presence the animals were milked, and brought to the laboratory in sealed cans. The acidity of the samples lay between 0·09 and 0·11 per cent. Thus the data analysed in this paper cover a period of about 8 months, from March to June (dry summer) and July to end of September (rainy season) when plenty of green pasture is available.

The animals in the dairy farm being managed under standard conditions, it was thought advisable to test random samples from animals under widely differing conditions of management. The City of Bangalore is largely supplied by producers who own hardly a few animals each. Animals in the City are stall-fed, while those in nearby villages go out to pasture. While rich owners feed their cattle with concentrates like cottonseed, groundnut cake, etc., poorer ones supply mostly hay and grass, and perhaps a little rice bran. Thus, about 30 samples each of cow and buffalo milk were collected at random from all classes of owners for examination.

The composition of a large number of samples collected were also estimated. The density (Celsius lactometer, 20° C.) and fat content (Gerber process) were used for computing the total solids (which closely agreed with the values of actual estimation) with the following formula for Indian milk:

$$T.S. = 0{\cdot}25\ (D\text{-}1000) + 1{\cdot}2\ F + 0{\cdot}66$$

The Refractive constant K, has been calculated according to the Lorenz and Lorentz formula:

$$\frac{n^2 - 1}{n^2 + 2} \times 1/d = K,$$

where n = R.I. (40° C.), d = density (20° C.) of milk.

More than 200 samples each of cow and buffalo milk have thus been analysed. The frequency distribution of R.I. and K are represented in Figures 1 and 2.

The relationship between S.N.F. and R.I. and between S.N.F. and K are illustrated in Figures 3 and 4.

Discussion of Data

Effect of Defatting Milk on R.I.—

The figures in Table I show that removal of fat by centrifuging causes no difference in R.I. of milk. This is to be expected as the fat is only a suspension in milk and forms no part of the solutes which contribute to the R.I.

Limits of R.I. and K. of Milk.—

While it is realised that a much larger number of analyses are to be completed before studying statistically the nature of the frequency curve, it is felt that the data collected so far are enough to warrant the fixing, at

least tentatively, of the extreme limits of variation of R.I. and K for genuine cow and buffalo milk.

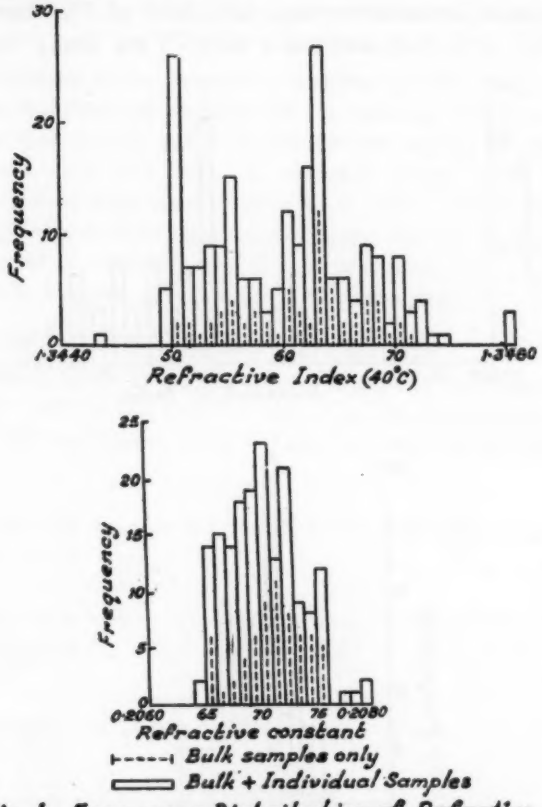

Fig. 1 Frequency Distribution of Refrative Index and Refractive Constant of Cow Milk.

Of all the samples examined about 50 per cent. were made up of individual samples and the rest of bulk samples. Among these about half were analysed in the dry months, March to June, and the rest in the months July to October when lush vegetation was available for cattle.

It is seen from Figs. 12 that the limits of R.I. of individual samples of cow milk normally extend from 1·3449 to 1·3480, buffalo milk from 1·3461 to 1·3500. The limits are, however, considerably narrowed down with bulk samples due to the ironing out of extremes of individuality by pooling milk. For cow milk these are 1·3450 to 1·3471, and for buffalo milk 1·3462

to 1·3487. It may here be repeated that these figures are inclusive of variations due to season, individuality and type of management (farm or village-bred cattle) and composition (cow milk, fat 2·15–7·1, total solids 11·47–16·00, S.N.F. 7·97–9·35 and ash 0·66–0·75 per cent.; buffalo milk, fat

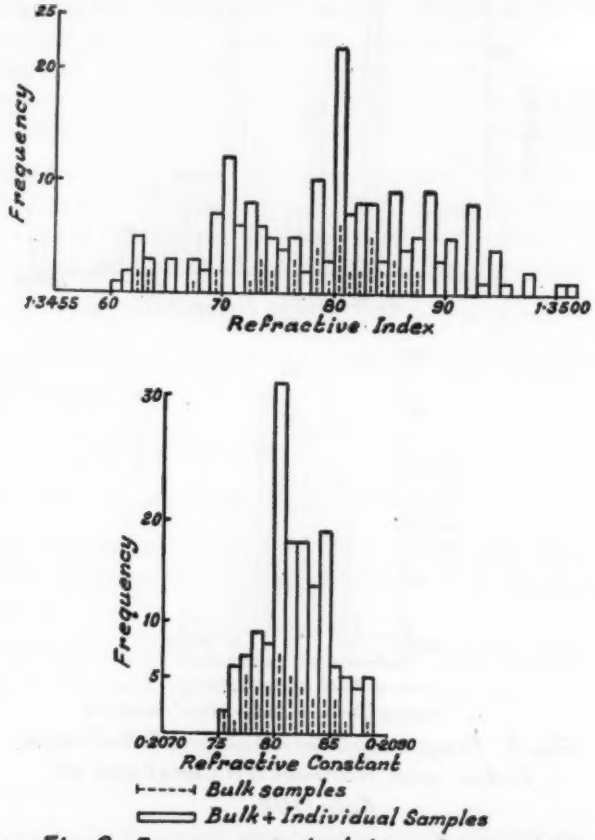

Fig. 2 Frequency Distribution of Refractive Index and Refractive Constant of Buffalo Milk

5·0–11·0, total solids 13·8–20·0, S.N.F. 8·03–11·3 and ash 0·70–0·85 per cent.). Within these limits the most frequently distributed value (the mode) is 1·3463 for cow and 1·3480 for buffalo milk. But it will be noticed that there are secondary maxima in each of the frequency diagrams. Analysis of the data (which are not given in detail due to shortage of space) in the light of seasonal variations indicate that the R.I. in the dry months has

an average and a mode distinctly lower than in the months when a plentiful supply of green herbage is available for consumption. Thus the mode is 1·3450 for cow and 1·3470 for buffalo milk in summer.

Refractive Constant.—The frequency diagram of this constant brings out the fact that the range of variation of the constant is not only considerably narrower than that of R.I. but is much less subject to changes due to external factors. For cow milk, K normally ranges from 0·2065 to 0·2075, and for buffalo milk from 0·2076 to 0·2088. The modes, 0·2070 for cow and 0·2080 for buffalo milk are also quite distinct from each other. Further, the range of K, unlike that of R.I., is practically the same for both individual and bulk samples, which is an added advantage.

Relationship between R.I. and K in cow and buffalo milk.—

It is noteworthy that although a certain degree of overlapping occurs in the ranges of R.I. of the two types of milk, K is characteristically different for each milk in the overlapping range. Table II brings out this fact clearly.

TABLE II

Relationship between R.I. and K in cow and buffalo milk

Cow		Buffalo	
Range of R.I. (40° C.)	K	Range of R.I. (40° C.)	K
1·3449–60	0·2065	1·3462–479	0·2076
49–60	66	67–80	77
49–63	67	65–86	78
50–66	68	70–87	79
54–73	69	63–92	80
49–67	70	71–93	81
53–69	71	72–94	82
50–70	72	78–97	83
54–72	73	72–501	84
62–70	74	82–494	85
43–80	75	84–92	86
72–78	79	80–97	87
		90–94	88

With the help of K and the corresponding R.I. it is thus possible to characterise the type of milk under examination with considerable certainty.

Relationship between S.N.F. and R.I. and K of Milk.—

Fig. 3 illustrates the approximate relationship between R.I. and S.N.F. of milk. Gross differences in S.N.F. are reflected, more or less, in corresponding changes of R.I. Elsdon and Stubbs (1929) observed a similar

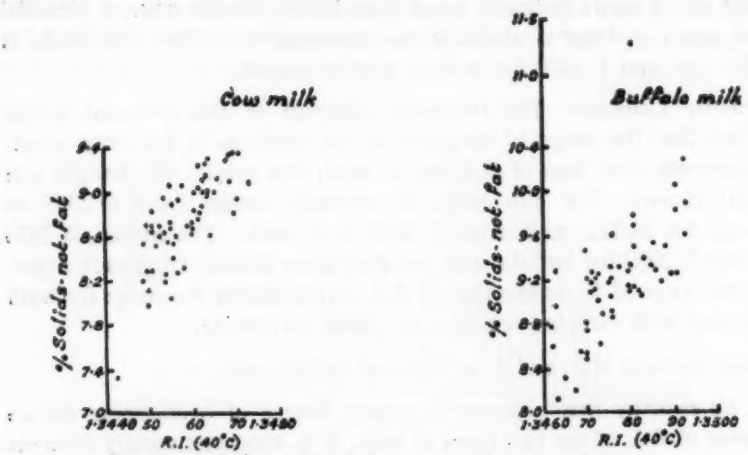

Fig.3. Relationship between Solids-not-fat and Refractive Index of Milk.

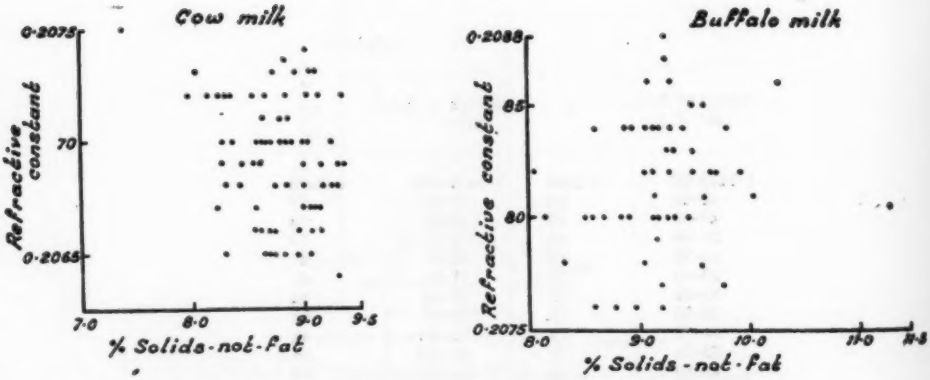

Fig. 4 Relationship between S.N.F. and K in Cow and Buffalo milk.

relationship between S.N.F. of milk and R.I. of milk-serum. But it is of advantage that no such relationship (Fig. 4) exists between S.N.F. and K. The figure shows that in spite of abnormal changes in the S.N.F. content of milk, K remains within normal limits.

TABLE III

Relationship between certain constants of milk low in S.N.F.

Density (20° C.)	S. N. F. %	R. I. (40° C.)	K
	Cow Milk		
1·0272	8·38	1·3457	0·2070
47	7·97	50	72
48	8·14	50	72
73	8·22	49	67
55	8·23	54	72
69	8·30	50	68
73	8·28	55	70
81	8·30	50	65
15	7·36	43	75
50	8·29	51	72
80	8·43	56	68
62	8·32	57	72
55	8·04	54	73
73	8·28	53	69
	Buffalo Milk		
1·0260	8·31	1·3465	0·2078
85	8·04	78	82
44	8·13	63	80
40	8·19	67	83

It can be seen from the above figures that samples of milk low in S.N.F. (with less than 8·5%) are usually associated with low R.I. and values of K considerably above the minimum for normal milk. It is perhaps possible that such a correspondence of the two constants is, as a rule, characteristic of genuine samples with low S.N.F. content.

In the light of the above data it is concluded that samples of cow milk with R.I. less than 1·3449 and K less than 0·2065 and buffalo milk with R.I. less than 1·3461 and K less than 0·2076 can be considered to be adulterated.

SUMMARY

A simple method of determining the refractive index of milk with the Abbè refractometer has been devised. About 10 c.c. of sample in a Gerber butyrometer is centrifuged for 5 minutes in an ordinary milk centrifuge. A few c.c. of the defatted milk is carefully collected without disturbing the fat layer and tested for R.I. It takes less than 30 minutes to test the R.I. of a dozen samples in this fashion; and these values represent, unlike those of milk-sera, the true refractive index of milk. From the density and R.I. the refractive constant, K, has been calculated.

The R.I. and K of more than 200 samples each of cow and buffalo milk have been tested over a period of 8 months. The limits of R.I. for cow milk lie between 1·3449 and 1·4480, the mode being 1·3463, and for buffalo milk between 1·3461 and 1·3500, the mode being 1·3480. K is distinct and lies within much narrower limits for each type of milk—cow milk 0·2065–0·2075, and buffalo milk 0·2076–0·2088. These limits, unlike those of R.I., are independent of the solids-not-fat content of milk. From the available data it is concluded that samples of cow milk with R.I. $< 1·3449$ and K $< 0·2065$, and buffalo milk with R.I. $< 1·3461$ and K $< 0·2076$ can be considered to be adulterated.

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ON *BEANIOPSIS RAJMAHALENSIS* GEN. ET SP. NOV., A NEW TYPE OF GYMNOSPERM FEMALE FRUCTIFICATIONS FROM THE JURASSIC OF BEHAR

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(With two Plates and two Text-figures)

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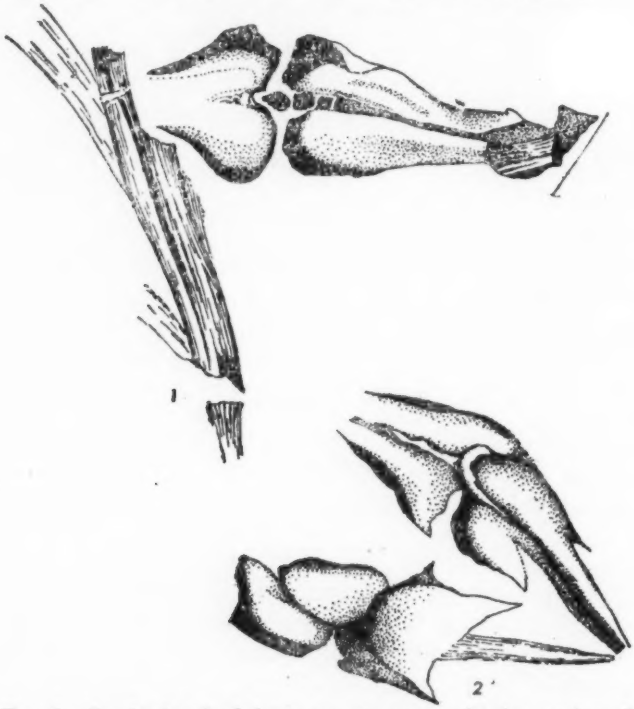
Introduction

The material on which this paper is based was collected at Onthea, during an excursion with Professor Birbal Sahni, F.R.S., in January 1942. The village of Onthea (in the Province of Behar) is situated at a distance of about 4 miles due south of Maharajpur railway station on the E.I.R. loop line between Sahebganj and Tinpahar (*Ganju, 1946, Text-Fig. 1*). Most of the forms described here were discovered on breaking hard siliceous rocks which apparently showed nothing on the exposed surfaces. The work was carried out at the Lucknow University under the guidance of Prof. B. Sahni. The figured material is preserved in the museum of the Department of Botany and Geology.

I wish to express my deep sense of gratitude to Prof. Sahni for his constant help and guidance given me readily throughout this work. I am greatly indebted to him for spending a good deal of his valuable time with me in discussing the nature and affinities of the new forms described in this and the two succeeding papers which will be published shortly. My thanks are also due to Mr. R. N. Lakhanpal, M.Sc., for kindly correcting the proofs of this paper.

This work records the find of a very interesting new type of Gymnosperm seed-bearing fructification, resembling in some respects the genus *Beania* from the Jurassic of Yorkshire, which is now regarded as the fructification of a true cycad, but having no clear affinity with that genus.



TEXT-FIG. 1. *Beaniopsis rajmahalensis* gen. et sp. nov. showing portion of the cone axis and a pair of seeds lying near it. The axis is longitudinally striated. On the left of the axis a sporophyll stalk is seen to arise. The two seeds are flattend out, the base of each is distinctly rounded and cordate and the micropylar end is elongated and flattened, giving the entire seed a rather flask like form. 0/18. × *Ca.* 12½.

TEXT-FIG. 2. *Beaniopsis rajmahalensis* gen. et sp. nov. Two pairs of seeds lying near each other. Each seed shows a very distinct median longitudinal groove continued from its base to apex. The micropylar ends of the two seeds in a pair point in a direction away from each other. 0/19. × *Ca.* 20.

DESCRIPTION

Beaniopsis gen. nov.

Generic Diagnosis

Female gymnospermous strobili of obscure affinity resembling Beania in their broad features, but differing in the fact that each sporophyll is expanded

distally into a spoon-like organ which bears upon its upper surface two divergent platyspermic ovules.

This genus is based upon seven well preserved specimens, all from Onthea. The specimens contain well preserved sporophylls attached to a central axis. The scales are stalked and each bears two ovoid sessile seeds placed distally in a broadly U-shaped depression of the sporophyll.

As regards the affinities, this genus is obviously a gymnosperm; the only groups within the gymnosperms to which any relationship at all can be suggested are the Cycadales and Ginkgoales, but even these comparisons must be regarded as distant and tentative. ***Beaniopsis*** bears some resemblance to the female fruits described by Carruthers under the generic name ***Beania*** from the Jurassic of Yorkshire. This genus was formerly regarded as a member of the Ginkgoales (*Seward, 1900, p. 276*), but Harris's recent work (*1941, pp. 82–97*) has shown that it resembles in all important characters the female cone of a typical living Cycad.

The specimens from Onthea, however, exhibit considerable differences from those described under ***Beania***. The differences lie in the size of the cone axis, in the shape and size of the sporophylls and seeds, in the mode of attachment of the sporophyll stalks to the cone axis, and in the mode of attachment of the seeds to the sporophyll. In consideration of these distinguishing features the specimens from Onthea have been placed in a separate genus which has been named ***Beaniopsis*** in order to indicate that it may possibly have some affinity with ***Beania***.

Specimens of this type have not so far been recorded from anywhere in India. Our knowledge of the genus is still necessarily imperfect, owing to the difficulty of obtaining complete specimens, but it may be that with the discovery of further material ***Beaniopsis*** may ultimately prove to be, like ***Beania***, a rare type of fossil Cycadean fructifications.

Beaniopsis rajmahalensis sp. nov.

(Pl. VII, Photos. 1–6; Pl. VIII, Photos. 7–11; Text-figs. 1, 2)

(Specimens 0/17–0/24)

Specific Diagnosis

Female cones about 1·5 cm. long, with a slender longitudinally striated axis less than 1 mm. thick. Sporophylls distant, attached alternately by means of slender stalks 2–3 mm. long and less than ·5 mm. wide, which usually arise at angles of 60°–70° from the cone axis. The apex of each sporophyll is differentiated into a spoon-like structure bearing two ovoid platyspermic

seeds, each about 1 mm. × 2 mm. The seeds are usually seen to lie with their principal planes in continuation with each other, but the orientation of this plane with reference to the median plane of the sporophyll stalk is still difficult to determine. The long axes of the two seeds in a pair diverge from each other at an angle varying from about 60° to 150°. The micropylar ends of almost all the seeds are embedded in the rock matrix and the broad chalazal end only is visible; at this end the principal plane of each seed is distinctly marked by a median longitudinal line.

Locality.—Onthea in the Rajmahal Hills. *Collection.*—Sahni and party (January 28, 1942). *Horizon.*—Rajmahal Series (Upper Gondwana).

Cone axis

The longest fragment has a cone axis about 1·5 cm. long, but it is quite likely that the original axis was somewhat longer. The rock is quite hard and fractures very irregularly, hence all efforts to develop the specimens failed. The axis in the largest specimen (0/21, shown in Photos. 1–3), which may be regarded as the type of the species, is hardly 1 mm. wide. The sporophylls arise from the axis in a spiral manner, at rather long intervals. The length of the internode is about 2 mm. Cones with attached sporophylls are preserved in only three specimens (0/21, 0/17, 0/23) shown in Photos. 1, 3, 4, 11. In all other specimens there are well preserved seeds but only fragments of the cone axis are available (Photos. 6, 7, 9).

The axis is longitudinally ridged but only two or three ridges are seen throughout its length. At places a ridge from the axis is seen to be continued for some distance into the stalk of a sporophyll. The sporophyll stalks arise from the axis at angles varying between 60° and 70°. A few stalks seem to arise at smaller angles. These are marked S in Photos. 1 and 4. These variations may be attributed to different modes of preservation rather than to any fundamental differences in the original specimens. As a rule we may take it that the sporophyll stalks arise from the axis at angles of about 65°.

Sporophyll stalk

The stalk of the sporophyll is 2 to 3 mm. long and less than $\frac{1}{2}$ mm. wide. It is straight and, like the axis, has a longitudinally wrinkled surface. These wrinkles are probably due to shrinkage on drying, as in the male flowers of a modern *Gingko*, and do not constitute a structural feature.

The distal end of the stalk enlarges to form a spoon like structure, which may be called the 'head'. This bears two seeds on its upper side, but the exact manner of attachment of the seeds is not quite clear. In several fairly

clear cases in which the pair of seeds is seen from the chalazal end, the median lines marking the principal planes of the two seeds lie in continuation with each other and in the same plane as the long axis of the sporophyll stalk. This seems to be the usual state of affairs. But there is at least one sporophyll (Photo. 2), on which the two seeds seem to lie side by side, obliquely, somewhat recalling the condition in *Cheirolepis*, although there are important differences of detail. The exact mode of attachment of the seeds, which is so important a diagnostic character, must unfortunately be left undecided until better preserved material comes to hand. As a rule the seeds are preserved on almost all the stalks and have not fallen off. This may indicate a rather young stage of development. Some pairs of seeds, however, occur detached from their sporophylls.

Seeds

The seeds are somewhat almond shaped and in preservation they assume peculiar forms which makes their interpretation somewhat difficult at first sight. The normal size of each seed is about 2 mm. by 1 mm. The micropylar ends of the two seeds in a pair point in a direction away from each other as shown in Text-Figs. 1, 2. The base of the seed is rounded and cordate; towards the micropylar end the seed becomes elongated and flattened so as to form a small tube-like structure, giving the entire seed a rather flask like form. Some of the rock matrix is enclosed between the two seeds at the base. By chipping the specimen, some of the seeds were fractured transversely but no details of the structure of the integument could be made out. The bicarinate integument is a marked feature of the seeds, the two carinæ being seen as very distinct median longitudinal ridges which are continued from base to apex. Not infrequently, however, the ridge is replaced by a groove (Photos. 7, 8) in which the rock matrix is seen wedged in. Sometimes the groove is so deep that at first sight it looks as if there are four seeds instead of two. If a fractured surface of a seed is examined carefully it is seen that this ridge is only a surface feature, probably representing the adhering remains of the integument which elsewhere has been broken off from the smooth surface of the nucule. The superficial nature of this ridge is seen in Photo. 10 where a seed marked S has been fractured transversely.

Some of the seeds which have been shed have been flattened out in preservation and the micropylar ends of the two seeds diverge from each other at a wider angle than usual. This character is quite common and almost all those seeds which have fallen are flattened in this manner. On looking at specimen 0/18 (Photos 9, 10; Text-Fig. 1) it seems as if this flattened seed is attached by means of its micropylar end but on a close

examination it becomes quite clear that the two seeds have become flattened out in preservation with their micropylar ends pointing away from each other. The ridge is clearly seen only in one seed, in the other it is present only at the base. These two seeds may or may not have belonged to the axis near which they lie. Another well preserved specimen bearing a pair of fallen seeds, flattened in the above manner, is seen in Photo. 6. Here the superficial nature of this ridge is quite evident.

The occurrence of this ridge may, however, be also explained in another manner. Some of the seeds of *Cycas* (*Seward, 1917, p. 25*) are at times flattened and have two prominent angles. Similarly three angled seeds have been found in *Ginkgo biloba* (*Seward and Gowan, 1900, p. 124*). It is quite likely that the seeds of ***Beaniopsis rajmahalensis*** were also two angled and this may explain the occurrence of the ridge.

Sometimes the sporophyll stalks seem to have become twisted through almost 180° so as to lie upside down and the 'head' of the sporophyll is seen worn away in such a manner as to make the chalazal ends of the seeds, with the attached sporophyll stalks, distinctly visible. This condition is clearly seen in Photo. 5, where two sporophyll stalks marked s1 and s2 have become twisted and the chalazal ends of the seeds are clearly visible.

The micropylar ends are embedded in the rock matrix but on examining closely the chinks around the seeds, it becomes obvious that the two micropyles point away from each other, the longitudinal axes of the seeds diverging at a wide angle which varies from 60° to 150°.

Comparisons

Of the other fossil remains which appear to offer comparisons with ***Beaniopsis*** special mention may be made of ***Beania, Ginkgo, Cheirolepis*** and ***Palissya.***

As far as the present data go, the closest resemblance appears to lie with ***Beania gracilis*** Carruthers. Until recently ***Beania*** was regarded as an extinct member of the Ginkgoales but it is now, in the light of Harris's recent work (*1941, p. 93*), to be regarded definitely as the fructification of one of the true Cycadales. If the resemblances with ***Beaniopsis*** are proved to be based upon a real affinity, then our fossils may also be considered to be the female cone of a cycad, although, of course, they are sufficiently distinct from it to be separated under a new generic name.

In ***Beaniopsis rajmahalensis*** the length of the cone axis is quite small (about 1·5 cm.), the size of the seeds likewise is smaller (usually 2 mm. by 1 mm.) and the sporophyll stalks arise from the axis at an angle less than a right angle.

On the other hand in ***Beania gracilis*** the mature cone is of considerable size (as long as 10 cm.), the seeds are correspondingly larger (the largest being 16 × 13 mm. and the smallest 7 × 7 mm.) and the sporophyll stalks arise from the axis at right angles. In ***Beaniopsis rajmahalensis*** the apex of each sporophyll is differentiated into a spoon-like structure bearing two ovoid platyspermic seeds on its upper side. The micropylar ends of the two seeds in a pair point in a direction away from each other and the principal plane of each seed is distinctly marked by a median longitudinal line. In ***Beania gracilis*** the head of the sporophyll stalks is usually flattened, and bears two oval seeds, which are placed parallel to the sporophyll stalks with their micropylar ends facing the cone axis.

If we compare ***Beaniopsis*** with ***Ginkgo***, we find that the differences are rather numerous. The female flower of ***Ginkgo biloba*** consists of an axis bearing two terminal sessile ovules, one on each side of the apex, the base of each ovule being enclosed by a small collar the nature of which has been differently interpreted by different authors. Some abnormal flowers have also been found in which the axis bears several ovules irregularly arranged. The peduncles bearing the ovules are greatly inclined to the axis. Such an arrangement, however, does not help us very much to relate ***Beaniopsis*** with ***Ginkgo***. In the ***Ginkgo*** flowers the ovules are terminal, the apex pointing outwards, on the other hand in ***Beaniopsis*** they are placed on the upper side of a spoon-like expansion of the sporophyll.

A comparison of ***Beaniopsis*** with ***Cheirolepis*** Schimper, and ***Palissya*** Endlicher does not lead us anywhere. Of these two ***Beaniopsis*** shows a nearer relation with ***Cheirolepis***. ***Cheirolepis*** (***Hirmer, 1936, p. 39***) has oval cones composed of a number of five-lobed cone scales each bearing two seeds. Each cone scale consists of a bract scale and a deeply lobed ovuliferous scale. The ovules lie nearly parallel to the bract scale with their micropyles facing the cone axis. We have seen that in ***Beaniopsis rajmahalensis*** there is at least one sporophyll on which the two seeds seem to lie side by side obliquely, suggesting a distant similarity with the arrangement in ***Cheirolepis***. This does not mean much when we know that such an abnormal position of the seeds in ***Beaniopsis*** is due to a twist in the sporophyll stalk.

Palissya (***Hirmer, 1936, p. 42***) has lax female cones. The cone scales are narrow and lanceolate with a pointed apex. Each cone scale bears 10–12 seeds in two rows and each seed has a characteristic cup like basal cupule. There is here no evidence of the double nature of cone scales as in ***Cheirolepis*** If we try to relate ***Beaniopsis*** with ***Palissya***, we are confronted with grave difficulties. Suppose we admit that the cone scale functions in the same

way as the sporophyll stalk and further that the basal cupule in *Palissya* is analogous to the cup shaped expansion of the distal end of the sporophyll stalk of *Beaniopsis*, we cannot account for the considerable difference in the number of seeds and their mode of attachment which is entirely different in the two.

On this point, however, a closer resemblance would seem to lie with *Stachyotaxus elegans* Nathorst (*Nathorst, 1908, p. 11; Hirmer, 1936, p. 42; Seward, 1919, p. 411*), in which the sporophylls arise approximately at right angles from the axis, each sporophyll consisting of a short and thick stalk, expanded distally into a scale like outgrowth with a pointed apex and bearing two seeds, each of which is placed in a cupule. But if we were to go into other details, we would find that here also the possibility of any close affinity totally fails.

In view of all these points, the real affinities of this extraordinary genus must be left undecided till some more material is available.

Summary

The interesting forms described in this paper under the new generic name *Beaniopsis* were found at Onthea in the Rajmahal Hills. Most of the specimens show well preserved female cones with distant sporophylls attached alternately to the cone axis by means of slender stalks. The stalk of the sporophyll is straight and like the cone axis its surface is longitudinally wrinkled. The distal end of each stalk is spoon shaped and bears two seeds on its upper surface. The exact mode of attachment of the seeds is not quite clear and must remain unsettled till better preserved material is found. Usually the seeds are placed in such a manner that the median lines marking the principal planes of the two seeds lie in continuation with each other and in the same plane as the long axis of the sporophyll stalk. The seeds are somewhat almond shaped with the base rounded and cordate, and the micropylar end is elongated and flattened. The micropylar ends of the seeds in a pair diverge from each other at a wide angle.

The genus is evidently a gymnosperm and the only groups to which a relationship can be suggested, with the present data in hand, are the Cycadales and less probably the Ginkgoales.

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Explanation of Plates

All photographs are untouched.

The figured specimens are preserved in the Department of Botany and Geology, Lucknow University.

Plate VII

Beaniopsis rajmahalensis gen. et sp. nov. (Photos. 1–6).

Photo. 1. Two female cones lying near each other. Each bears a number of alternately attached sporophylls. The sporophyll stalks marked S are seen to arise from the cone axis at a smaller angle than usual. 0/21. × 2.

Photo. 2. The same specimen enlarged to show the nature of cone axis and of the sporophyll stalk. On one sporophyll (s) the two seeds seem to lie side by side, obliquely. 0/21. × 4.

Photo. 3. Part of a female cone. The cone axis shows longitudinal striations distinctly at one or two places. 0/21. × 2.

Photo. 4. A well preserved female cone bearing alternately attached sporophylls. The sporophyll stalk marked S is seen to arise from the cone axis at a smaller angle than usual. 0/17. × 2.

Photo. 5. The same specimen enlarged. The sporophyll stalks marked s1 and s2 have been twisted, the head of the sporophyll is seen worn away and the chalazal ends of the seeds with the attached sporophyll stalks are clearly visible. 0/17. × *Ca.* 3½.

Photo. 6. A pair of seeds flattened out and exposed by their chalazal ends. The micropylar ends of the two seeds seem to diverge from each other at a rather wide angle. 0/17. × 13.

PLATE VIII

Beaniopsis rajmahalensis gen. et sp. nov. (Photos. 7–11).

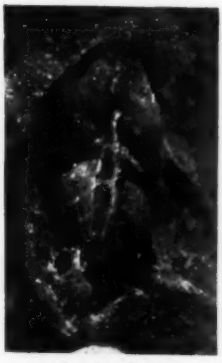
Photo. 7. Two pairs of seeds lying side by side. Each seed shows a distinct median longitudinal groove continued from its base to apex. In the upper part of this photograph fragments of cone axis are seen. 0/19. × 2.

Photo. 8. The same specimen enlarged. Towards the top left of this photograph the round and cordate base of a seed is seen rather well preserved. × *Ca.* 6.

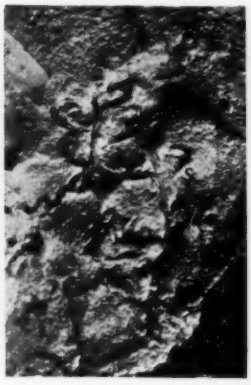
Photo. 9. Fragments of cone axis with a few attached sporophylls. The position of a pair of well preserved seeds lying near an axis is indicated by means of an arrow. 0/18. × 2.

Photo. 10. A portion of the same specimen enlarged to show the pair of seeds referred to above. Each seed is flattened and shows a distinct median longitudinal ridge continued from its base to apex. The micropylar end of the seed is flattened out and the base is rounded and cordate. Towards the top left of this photograph, a seed marked S, is fractured transversely and reveals the superficial nature of the ridge. × *Ca.* 6.

Photo. 11. Fragment of a female cone. 0/23. × 2.

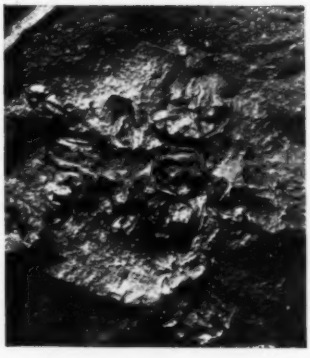


6. (×13)



1. (×2)

2. (×4)



3. (×2)

5. (×ca. 3½)

4. (×2)

FIGS. 1—6. *Beaniopsis rajmahalensis*

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8. (×ca. 6)

9. (×2)

7. (×2)

10. (×ca. 6)

11. (×2)

FIGS. 7—11. *Beaniopsis rajmahalensis*

ONTHEANTHUS POLYANDRA GEN. ET SP. NOV., A NEW TYPE OF FOSSIL GYMNOSPERM MALE FRUCTIFICATIONS FROM THE RAJMAHAL HILLS

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Received August 25, 1946
(Communicated by Prof. B. Sahni, F.R.S.)

(With four Plates and four Text-Figures)

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Introduction

The fossils here described were collected on 28th January 1942, at Onthea, a village in the province of Behar, by a party of research students of Lucknow University led by Professor Birbal Sahni, F.R.S. The author, then a research student was also a member of the party. The locality of Onthea was previously known to earlier investigators like Oldham, Morris, and Feistmantel. The exposure of the plant-bearing strata is not far from the village (*Ganju, 1946, Text-Fig. 1*). Almost all the fossils were collected from beds *in situ*, and, therefore, there is no doubt that they belong to the Rajmahal Series. The different forms were found on breaking hard, fine grained, siliceous rocks. A few of the 'flowers' were found by Dr. R. V. Sitholey while he was breaking some pieces of the big slab (*Ganju, 1946, Introduction*). I am

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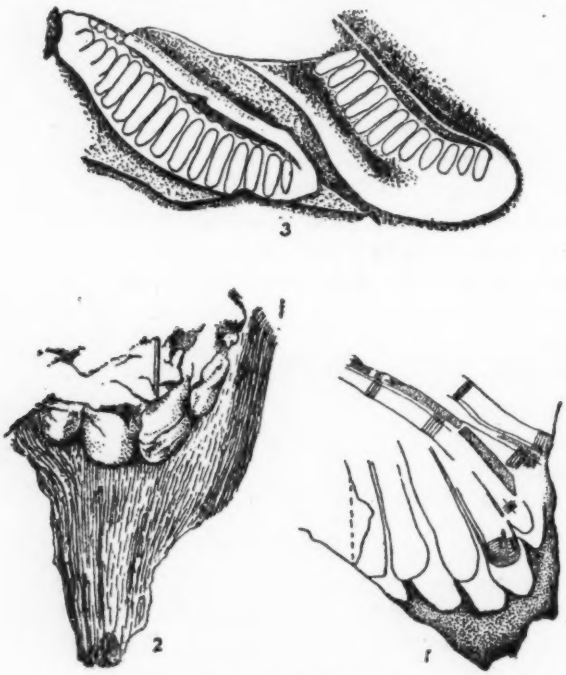
extremely grateful to Dr. R. V. Sitholey who has been kind to hand over these specimens to me for description.

The entire work was carried out under the close supervision of Professor Sahni. The figured specimens are kept in the museum of the Department of Botany and Geology, Lucknow University.

It is matter of very great pleasure to express my indebtedness to Professor Sahni for the ungrudging help and valuable guidance he has been constantly giving me. It is needless to say that without his suggestions it would have been well nigh impossible for me to bring up this work to its



Ontheanthus polyandra gen. et sp. nov.

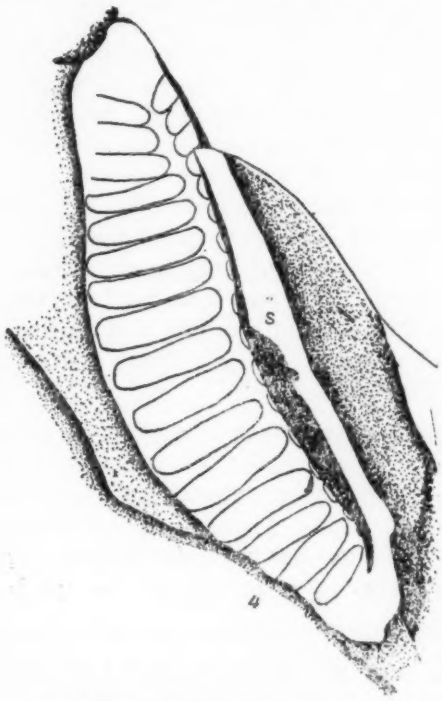
TEXT-FIG. 1. A number of microsporophylls viewed from their adaxial surface and lying on the inner side of a portion of the perianth. Each sporophyll is divided into two parts, (i) a very short sterile basal portion broadly rounded at its lower end and continued distally into (ii) a long and narrow very gradually tapering scale. 0/30. × 1¾.

TEXT-FIG. 2. Basal portions of four or five sporophylls lying on the inner surface of the perianth. 0/45. × 2¾.

TEXT-FIG. 3. Lateral view of two uppermost sporophylls of the specimen shown in Photo. 10. These sporophylls have become folded longitudinally along the middle line so that the two rows of synangia make an acute angle with each other. 0/72. × *Ca.* 6¾.

present form. I am again indebted to Mr. Lakhanpal for the trouble he took in seeing the proofs of this paper.

An extremely interesting and an altogether new type of a gymnosperm male fructification, is described in this paper. It is not possible to express



Ontheanthus polyandra gen. et sp. nov.

TEXT-FIG. 4. Camera lucida sketch of the uppermost sporophyll of the specimen shown in Photo. 10. Here a strip of the abaxial sterile tissue is clearly seen to bear on its upper (adaxial side) the two rows of synangia which have been brought into view by the removal of the abaxial sterile tissue except for one narrow strip marked s. 0/72. × 14.

any definite views about the real affinities of this form, but it seems to be a male 'flower' of some unique type of Bennettitales.

DESCRIPTION

Ontheanthus gen. nov.

Generic diagnosis

Male flowers, probably Bennettitalean, with numerous elongated microsporophylls crowded round an elongated central receptacle which is enclosed

within a cup-like perianth. Each sporophyll bears two contiguous rows of crowded transversely elongated synangia.

Under this genus are included some gymnosperm fructifications of a very interesting new type which were found only at Onthea. The only species of this genus, *Ontheanthus polyandra* sp. nov., is represented by at least fifteen specimens. All the specimens are incomplete but a provisional reconstruction of the numerous fragments suggests that here we have an altogether new type of male fructifications probably belonging to one of the Bennettitales. A large number of elongated microsporophylls, rounded at the base and gradually narrowing towards the distal parts, are attached in a crowded manner all round a thick elongated receptacle. The sporophylls bear numerous narrowly oblong sporangium-like organs placed transversely in two contiguous rows. Some of these sporangium-like bodies show definite signs of partition into chambers. They must, therefore, be regarded as micro-synangia, although no spores have yet been found in them. The entire receptacle with its mass of sporophylls is enclosed in single cup-like perianth. The internal surface of this perianth and the surface of the receptacle are peculiarly wrinkled.

No definite views can be expressed regarding the affinities of this fossil. As will be shown later, this fructification seems to be a male 'flower' of some peculiar type of Bennettitales resembling in some respects the genus *Cycadeoidea*.

Ontheanthus polyandra sp. nov.

(Pl. IX, Photos. 1–4; Pl. X, Photos. 5–7; Pl. XI, Photos. 8–11; Pl. XII, Photos. 12–14; Text-Figs. 1–4)

(Specimens 0/30–0/37, 0/39–0/46, 0/71–0/75, 0/80, 0/103)

Specific diagnosis

Flower pedunculated, at least 7 cm. long. Peduncle about 1 cm. thick. Perianth deeply funnel shaped, about 5·5 cm. long and 3·3 cm. in the broadest part, with the inner surface longitudinally wrinkled. Receptacle at least 3·5 cm. long, 2 cm. in its broadest middle portion, gradually narrowing towards the base and apex. The surface of the receptacle is longitudinally corrugated in the same manner as the inner surface of the perianth. Androecium exserted. Sporophylls very numerous, at least 120 but probably many more in each flower, attached in a densely crowded manner all round the receptacle throughout its length and overlapping each other. Each sporophyll is attached by a thick and broadly rounded sterile base about 3 mm. × 1 mm. This is continued distally into a long and narrow, very gradually tapering scale about 2·5 cm. long, and

about 3 mm. to 1·5 mm. broad, which bears on its adaxial surface two closely set rows of narrowly oblong synangia. The two rows of synangia are contiguous with each other, being separated only by a narrow median line. They cover the whole upper surface of the scale, excluding only a very narrow marginal strip of the sterile part of the sporophyll on either side. The synangia in each row are also contiguous; those in the broad basal part of the scale (next to the sterile base) are about 1·4 mm. × ·5 mm., those placed more distally about ·7 mm. × ·3 mm. Some of the synangia show clear signs of being divided, as in Cycadeoidea, into a number of chambers which lie on the two sides of median partition, Spores not seen.

Locality: Onthea (about four miles due South of Maharajpur railway station), in the Rajmahal Hills, Behar. *Collection*: Sahni and party (January 28, 1942). *Horizon*: Rajmahal Series (Upper Gondwana).

General Structure of the Flower

The above diagnosis is based upon a reconstruction of about fifteen specimens, none of which is complete. The most complete flower, which is to be regarded as the type specimen, is shown in Photo. 1. This represents a longitudinally fractured flower, the other half of which was broken while splitting the rock. The portion preserved measures about 6·5 cm. long and 3·3 cm. wide. It shows an outer continuous cup-like envelope (*c*) serving as a perianth. The distal part of the perianth is not preserved and, therefore, it is impossible to say whether it was a continuous structure or lobed as in most Bennettitalean flowers.

That the fracture through this flower has passed somewhat tangentially is evident from the fact that we see a number of sporophylls (about eight or nine), placed longitudinally, even in the middle of the specimen (Photo. 1). These must have been attached on the side of the receptacle facing away from the reader, for they all present their adaxial (that is, synangium-bearing) surface. The sporophylls attached along the right and left sides of the section are only seen cut through their sterile swollen bases, which thus appear like two longitudinal series of lobes.

It is not easy to estimate the thickness of the receptacle in a tangential section, but it appears to have been at least 2 cm. thick, and this estimate agrees more or less with what we find in Photo. 4.

The entire hollow of the perianth must have been filled up by the receptacle and its attached microsporophylls. The distal parts of the sporophylls must have expanded out from the perianth, like the exserted stamens of a modern flower. The internal surface of the perianth and the surface of receptacle are longitudinally wrinkled throughout (Photos. 1, 4).

Receptacle, Peduncle, Mode of Attachment of Flower

In Photo. 4 we see two flowers lying almost parallel with each other, possibly attached to one and the same axis which, however, is not preserved. In each of these the flowers longitudinally wrinkled lower half of the receptacle is seen gradually narrowing downwards into a thin cylindrical peduncle, especially well seen in the right-hand flower. Attached on the upper part of each receptacle we see (especially in the left-hand flower) the swollen basal parts of a few sporophylls which are seen continued distally into their narrrow fertile portions.

Number of Sporophylls in the Flower

The number of microsporophylls in each flower must have been very large indeed, to judge by their densely crowded arrangement. Some idea of their number may be gained by examining Photos. 1, 2, 4, 5. In the middle region of Photo. 1 we see, as already stated, about eight or nine sporophylls placed in a transverse row roughly parallel to each other. The number of sporophylls met with in passing along a vertical section (as represented by the lobes at the right side of the photograph) is about eight. Assuming that the other half of the flower also contained the same number, if not more, we may take it that on an average there were, in all, about 128 to 144 such scales in each flower.

Roughly the same sort of estimate is arrived at by examining Photo. 2 which also represents a tangential section through a flower, although here the perianth is only preserved on one side (*p*). Here too we find that in passing transversely across this photograph eight or nine sporophylls are met with, placed parallel to each other as in Photo. 1. In the lower part of the photograph the swollen bases of a few of these sporophylls are seen. The number of sporophylls traversed in passing from below upwards is not clear, but if we compare Photo. 10 it is evident that this number must be not less than seven.

Photo. 4 illustrates the considerable length which the receptacle must have attained, and also shows (see left-hand flower) that it was fertile even in its narrow distal portion which here is clearly seen bearing a vertical series of thick sterile lobes tapering upwards into their narrow fertile scales.

Form and Structure of the Microsporophylls

The microsporophylls reach a length of about 2·5 cm. Each sporophyll is divided into two parts, (i) a very short sterile basal portion about 3 mm. × 1 mm. broadly rounded at its lower end and concave along its upper margin. This is continued distally into (ii) a long and narrow very

gradually tapering scale, about 2·5 cm. long and 3 mm. to 1·5 mm. broad. It is likely that the scales reached a considerably greater length and projected well beyond the perianth. These bear on their adaxial surface two closely set rows of narrowly oblong synangia. It is by the short sterile basal portion that the sporophyll is attached to the receptacle. The longitudinal section in Photo. 1 shows clearly on the right-hand side the attachment of seven or eight sporophylls of which only their basal portion is visible. The same kind of structure less clearly preserved, is seen in the left-hand side of the photograph. In Photo. 5 (Text-Fig. 1), we see four or five sporophylls viewed from their inner (adaxial) surface, and lying on the inner side of a portion of the perianth. The same specimen is shown slightly enlarged in Photo. 6 and still further enlarged, to show the details, in Photos. 7, 8 and 12. In several of the sporophylls the sterile marginal strips are seen as thickened ridges extending along the two sides of the sporophyll, especially in their basal portions. Covering a portion of the third sporophyll from the right, are detached strips of the abaxial sterile portions of one or two sporophylls which lay above them (that is, in a position internal to them with respect to the receptacle), and were broken off with the counterpart.

The basal parts of the scales are seen to overlap one another. Those which pass along the plane of section are seen complete. The microsporophylls are closely packed leaving no space between them.

In the specimen shown in Photo. 1 it looks as if the sporophylls are attached to the inner surface of the perianth but this appearance must be deceptive. This flower has accidentally been fractured tangentially, and the portion of the perianth on which the sporophylls appear to be attached is seen from the inner surface to which the sporophylls have remained adhering. A somewhat similar specimen is seen in Photo. 2 which has already been described above.

In Photo. 10 we see a lateral view of a row of about seven sporophylls attached to the receptacle (*r*) and viewed from their abaxial sides. Of these the three uppermost microsporophylls are further enlarged in Photo. 11 to show clearly the form and arrangement of the synangia. The synangia have been brought into view by the removal of the abaxial sterile tissue of some of the sporophylls except for one narrow strip marked *s* (see also Text-Figs. 3, 4). Specimen 0/33 (Photo. 3) which in its general features is comparable with Photo. 5, shows relatively narrower and longer sporophylls, lying on the inner surface of a portion of the perianth. Specimen 0/45 (Photo. 9; Text-Fig. 2) shows the basal portions of four or five sporophylls lying on the inner surface of the perianth.

The Microsynangia

The microsynangia are contained in numerous narrowly oblong sac-like organs arranged transversely in two dense rows, one row on either side of a median line. They lie quite flat, usually making an angle of 90° with this line (Photo. 12). Some sporophylls have become folded longitudinally along the middle line so that the two rows of synangia make an acute angle with each other (Photos. 7, 11; Text-Figs. 3, 4). As a rule, however, the adaxial surface of the sporophyll is flat so that all the synangia lie in one plane. Text-fig. 3 has been drawn on an enlarged photograph of the specimen shown in Photo. 10. These are the two uppermost sporophylls of the row. Here a strip of the abaxial sterile tissue is clearly seen to bear on its upper (adaxial side) the two rows of synangia which make an acute angle with each other. Text-Fig. 4 shows a camera lucida sketch of the uppermost sporophyll still further enlarged.

Each synangium is about ·7–1·5 mm. long and ·3 to ·5 mm. broad, so that in a longitudinal distance of 1 mm. two or three of them are traversed. In specimens 0/71, 0/30 and 0/31 (Photos. 2, 12 and 13 respectively) some of the synangia when carefully examined with the help of a pocket lens from all directions seem to be divided into a number of chambers along a faint median line, somewhat like the synangia on the micro-sporophylls of *Cycadeoidea*. Some other specimens, however (Photo. 11), only reveal the presence of a number of small rounded raised areas probably marking the position of the individual sacs of the synangia. Attempts to obtain spores have, however, completely failed. Portions of synangia were broken off for maceration. Both concentrated nitric and hydrochloric acid were tried but the rock did not disintegrate. A careful examination of the exposed surface of the synangia under the high power of a microscope also did not reveal the presence of any spores.

Discussion

Nature of the Parent Plant: Reconstruction

It is difficult to say anything definite about the nature of the parent plant and the manner in which these flowers were borne on it. Fortunately, however, a specimen (No. 0/73) has been found which enables us to gain some idea of the kind of stem on which the flowers were attached. This specimen shown in Photo. 14 shows a branched axis about 10 cm. long and 8 mm. broad, wrinkled in just the same fashion as the inner surface of the perianth and the exposed surface of the receptacle of *Ontheanthus polyandra* sp. nov. Towards the left side (at *f*) is preserved a fragment of a flower of

the same species, attached to a short stalk and bearing a number of well preserved microsynangia of the characteristic type already described. The angle at which this stalked flower lies with respect to the long axis just mentioned is such as to leave no doubt that the stalk was continued downwards and was fixed to the main axis near the point marked *x*. Higher up on the same axis another branch is seen to arise towards the right. This branch is again wrinkled in the same manner as the main axis, and must also have borne a flower of *O. polyandra*, but of this flower there is no direct evidence, because the branch is broken off distally.

In another specimen (No. 0/42) shown in Photo. 4, which has already been described above, two flowers are placed nearly parallel to each other. These may have been attached below to one and the same branch bifurcating near the apex, each branch ending in a flower.

Affinities

The flower described here does not resemble, even in broad features, any genus hitherto known, hence it is extremely difficult to explain its affinities. It is an entirely new type of fructification and most of its features are so unique as to make its study very interesting. For the same reason its interpretation is a matter of some difficulty, with the result that the morphology of the flower is in some respects still rather obscure.

Although a perianth is present, a reference to angiosperms is obviously out of the question. Knowing that these flowers come from beds of Jurassic age, the only groups which can come into consideration are the gymnospermous phyla Coniferales, Cycadales and Bennettitales. A comparison with the Coniferales and Cycadales is ruled out because of the presence of a definite cup-shaped perianth and the structure of the sporophylls. With the Bennettitales, however, these flowers do show at least some broad features in common which suggest that it is a male fructification of some unusual type either belonging to or related to this diversified phylum which is so strongly represented in the Rajmahal flora. Let us consider these points in some detail.

Firstly, the general organisation of *Ontheanthus polyandra* is just like that of a Bennettitalean flower. There are numerous sporophylls attached to a thick receptacle which is enclosed by a perianth.

Secondly, the structure of the sporophylls is strongly suggestive of a Bennettitalean affinity, the presence of synangia divided into chambers as in *Cycadeoidea*, and their biseriate arrangement, being important points specially worthy of consideration.

As we shall see, however, there are also equally important points of difference.

As regards the feature in which *Ontheanthus* differs from the Bennettitales there are three: (i) The mode of attachment of the microsporophylls, which instead of being placed in a single whorl are disposed spirally in a densely crowded manner on a thick elongated receptacle. (ii) The absence of a basal cup-like portion of the androecium such as we find in *Williamsonia, Cycadeoidea,* and other Bennettitales. (iii) The presence of a cup-like perianth.

The nature of the associated vegetative remains, *e.g.,* of leaves, also sometimes helps in indicating the affinities of a new type of flower, but one must not attach too much importance to mere associations. The only leaves found in close association with these flowers are those of the *Ptilophyllum acutifolium* type. We know that the leaves known as *Ptilophyllum* cf. *cutchense* were almost certainly the foliage of *Williamsonia Sewardiana,* but the nature of the fructifications borne by the parent plants of the other Indian *Ptilophyllums* are still unknown. It is not impossible that one of the component forms of the aggregate "species" *P. acutifolium* may have been the foliage of the plant which bore the flowers here described as *Ontheanthus polyandra.* It is true that the branched vegetaive axis (Photo. 14) which bore the flower of *Ontheanthus* is wrinkled throughout and shows no trace of a rhomboid leaf scar such as a *Ptilophyllum* might be expected to leave on a stem. But it is not inconceivable that the flowering shoots of *Ontheanthus* bore only small scale-like leaves while the mature vegetative stem carried foliage leaves of the *Ptilophyllum* type. At the same time it must be confessed that all this is mere speculation: the foliage of *Ontheanthus* is still unknown.

No evidence of the female parts of *Ontheanthus* has been yet found, although of the male flowers as many as fifteen specimens have been found. It is not likely that any ovulate organs were attached to the receptacle of the species here described as *O. polyandra,* because the receptacle bearing the microsporophylls is quite thick and fills the entire hollow of the perianth, leaving no room for the attachment of female organs. But some *Bennettitales* were certainly unisexual and others hermaphrodite. It is, therefore, quite probable that in this new form we have an unisexual type of flower and that the female flower of this type is still to be discovered. A further search at Onthea may reveal its presence there.

Considering all these facts, we evidently have in *Ontheanthus* an entirely new and a distinct genus of very great interest which, while showing

Bennettitalean affinities, at the same time exhibits peculiar features of its own. In the form and arrangement of sporophylls, and in the structure of synangia this new genus may be compared to the genus *Cycadeoidea* in some of its broad features. But in the densely crowded spiral mode of attachment of the sporophylls, which moreover are placed all over the extensive surface of a thick and elongated receptacle it is, so far as we know, a unique type among the Bennettitales. The cup-like perianth is also a unique feature.

The fossils described here have been given the new name *Ontheanthus polyandra* gen. et sp. nov. This name has been chosen in order to denote a male flower from Onthea containing numerous microsporophylls. This locality was known to Oldham as early as in 1863 (*see Oldham and Morris, 1863, Preface, p. xv*) and to Feistmantel in 1877 (*Feistmantel, 1877, p. 6*), but it is only in recent years that extensive search at this locality has begun to reveal a number of interesting new types of plants. Among these *Beaniopsis* (*Ganju, 1946 a*), *Ontheanthus* and *Ontheostrobus* (*Ganju, 1946 b*) are three of the most important forms (*Ganju, 1944, pp. 76–77*).

Summary

The new type of male fructification described in this paper under the name *Ontheanthus polyandra* is fairly common at Onthea. The 'flower' consists of a short peduncle and a deeply funnel-shaped perianth. The receptacle is about 2 cm. thick and must have been at least 3·5 cm. long. The exterior of the receptacle and the inner surface of the perianth are wrinkled. The sporophylls are numerous and crowded throughout the length of the receptacle. Each sporophyll consists of a short sterile broadly rounded basal portion, which passes distally into a long and a narrow scale tapering very gradually and projecting beyond the perianth. These microsporophylls bear on their adaxial surface two closely set rows of narrowly oblong synangia arranged transversely, one row on either side of a median line. The synangia lie quite flat, usually making an angle of 90° with this line, but some sporophylls have become folded longitudinally in a peculiar manner with the result that the two rows of synangia make an acute angle with each other.

It is rather difficult to say anything definite regarding the nature of the parent plant and the manner in which these flowers were borne on it. It is equally difficult to be certain about the real affinities of such an unusual type of fructification. All that can be said at present, after taking into consideration the broad features only, is that our flower is a male fructification

probably of one of the Bennettitales. In this group the only plausible comparison, in respect to the form and the arrangement of sporophylls, is with the genus *Cycadeoidea.*

This find of an interesting new type of fructification is a notable addition to other interesting forms already known from the Jurassic beds of the Rajmahal Hills. The Bennettitales were at the height of their development during the Jurassic period and *Ontheanthus polyandra* comes from beds definitely known to be of Jurassic age.

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Explanation of Plates

All photographs are untouched. They are of natural size except where the magnification is given.

The figured specimens are preserved in the Department of Botany and Geology, Lucknow University.

Plate IX

Ontheanthus polyandra gen. et sp. nov. (Photos. 1–4).

Photo. 1. A longitudinally fractured flower. The outer continuous cup-like envelope (*c*) is the perianth. On the right-hand side the attachment of basal portions of seven or eight sporophylls is visible. The same kind of structure, less clearly preserved, is seen in the left-hand side of the photograph. 0/71.

Photo. 2. Portion of a flower fractured tangentially. The perianth (*p*) is only preserved on one side. A number of microsporophylls parallel to one another, can be clearly seen. This flower lies near the one shown in Photo. 1. 0/71. × *Ca.* 1½.

Photo. 3. The sporophylls in this flower are relatively narrower and longer. 0/33. × 1¾.

Photo. 4. Two flowers lying almost parallel with each other. A portion of the peduncle is especially well seen in the right-hand flower. The longitudinal wrinkles on the surface of the receptacle are clearly visible. 0/42.

Plate X

Ontheanthus polyandra gen. et sp. nov. (Photos. 5–7).

Photo. 5. A number of closely packed microsporophylls viewed from their adaxial surface and lying on the inner side of a portion of the perianth. The basal parts of the sporophylls are seen to overlap one another. 0/30.

Photos. 6, 7. The same specimen enlarged to show the structure of microsporophylls. The sterile marginal strips are clearly seen as thickened ridges extending along the two sides of the sporophyll. Some sporophylls have become folded longitudinally along the middle line so that the two rows of synangia make an acute angle with each other. Photo. 6 × 2; Photo. 7 × *Ca.* 3.

Plate XI

Ontheanthus polyandra gen. et sp. nov. (Photos. 8–11)

Photo. 8. Specimen shown in Photo. 5 enlarged to show the broadly rounded short sterile basal portion of the sporophyll. The microsynangia are contained in narrowly oblong sac-like organs arranged transversely in two dense rows, one row on either side of a median line. × 4.

Photo. 9. The basal portions of four or five sporophylls lying on the inner surface of the perianth. 0/45. × 1¾.

Photo. 10. Lateral view of a row of about seven microsporophylls attached to the receptacle (*r*), viewed from their abaxial sides. 0/72. × *Ca.* 2.

Photo. 11. The same specimen enlarged to show the three uppermost microsporophylls. The synangia have been brought into view by the removal of the abaxial sterile tissue, except for one narrow strip marked *s*. The sporophylls have become folded longitudinally along the middle line so that the two rows of synangia make an acute angle with each other. × 4.

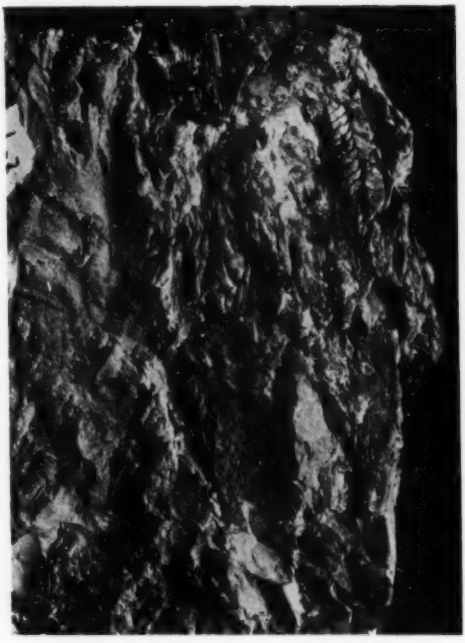
PLATE XII

Ontheanthus polyandra gen. et sp. nov. (Photos. 12–14).

Photo. 12. Part of the specimen shown in Photo. 5 enlarged. On some microsporophylls the microsynangia lie quite flat, usually making an angle of 90° with the median line. × *Ca.* 5.

Photo. 13. Two sporophylls lying side by side. Some of the microsynangia show rather clearly the presence of a faint median line. 0/31. × 4¾.

Photo. 14. A branched axis which bore the flowers. At *f* is preserved a fragment of a flower attached to a short stalk which was fixed to the axis near *x*. Another branch is seen to arise at *r*. 0/73. 9/10 Nat. size.



4.

3. (×1¾)

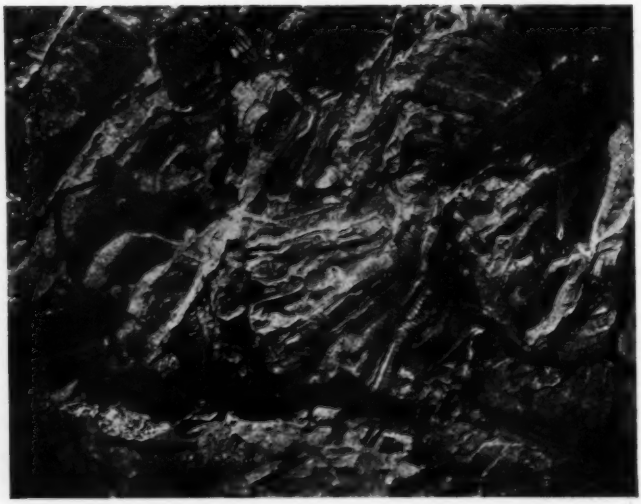
1.

2. (ca. 1½)

FIGS. 1—4. *Ontheanthus polyandra*

5.

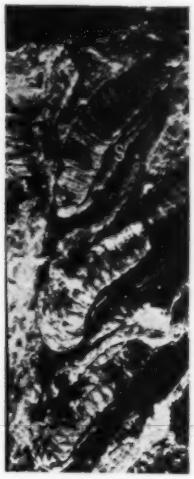
7. (×ca. 3)



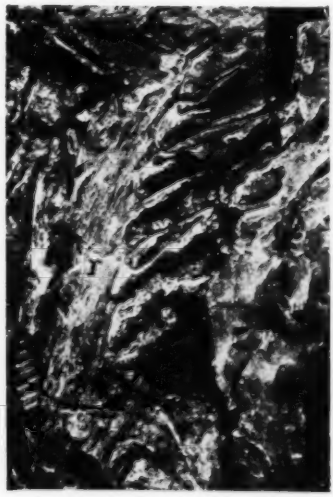
6. (×2)

FIGS. 5—7. *Ontheanthus polyandra*

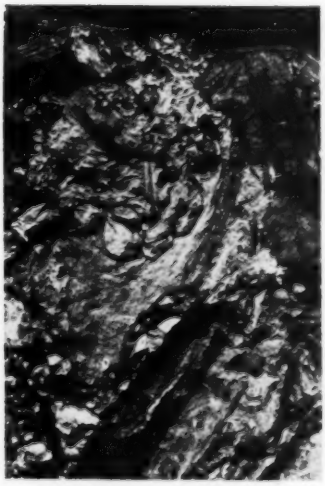
8. (×4)



11. (×4)

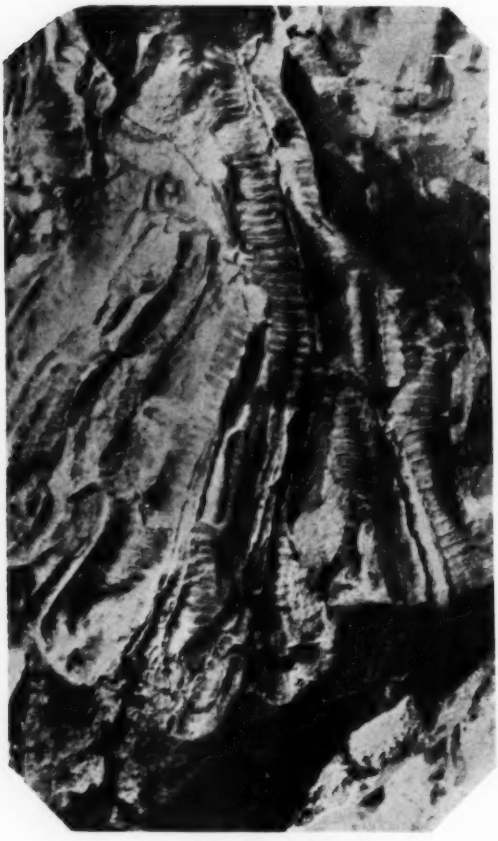


10. (×ca. 2)



9. (×1¾)

FIGS. 8—11. *Onthcanthus polyandra*



12. (×ca. 5)

13. (×4¾)

14. $\frac{9}{10}$ Nat. size

FIGS. 12–14. *Ontheanthus polyandra*

ONTHEOSTROBUS SESSILIS GEN. ET SP. NOV., A NEW TYPE OF SEED-BEARING GYMNOSPERM FRUCTIFICATIONS FROM THE JURASSIC OF ONTHEA IN THE RAJMAHAL HILLS

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(With two Plates)

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Introduction

The interesting material which forms the subject-matter of this paper was found at Onthea on 28th January 1942, when also the forms described earlier (*Ganju, 1944, pp. 76-77, 1946, 1946 a, 1946 b*) were collected. Onthea has now turned out to be a very interesting and useful place for the study of fossil plants. The forms described in all these four papers were found in a single day's field work. One can easily imagine the wealth of material that the fossiliferous outcrop at Onthea (*Ganju, 1946, Text-Fig. 1*) may be expected to yield on a detailed study.

Some of the forms here described were seen on the exposed weathered surface of the specimens, while some were discovered only on breaking the familiar kind of hard grey siliceous rocks so commonly found at Onthea. Dr. R. V. Sitholey found a few specimens while breaking the big slab, a detailed reference to which has been given in an introduction to the first paper (*1946*). I am very thankful to Dr. Sitholey for kindly giving me these specimens for description.

The work has been carried out, as usual, under the guidance of Professor Birbal Sahni, F.R.S., to whom I owe sincere thanks for helping me to plod through many of the difficulties that so often confront one in a new field of research. I have to thank Mr. R. N. Lakhanpal for correcting the proofs of this paper.

An extremely interesting new type of seed-bearing gymnosperm fructification is described in this paper. It is not possible yet to say anything definite about the real affinities of this form, but at first sight a distant comparison with the Bennettitales is suggested. The absence of interseminal scales, however, remains an obstacle in including this genus within that phylum. For the present, therefore, the systematic position of this type of fructification must remain unsettled.

Description

Ontheostrobus gen. nov.

Generic diagnosis

Megastrobilus consisting of an axis bearing numerous crowded sessile seeds on all sides. Seed orthotropous, seated in a shallow saucer-shaped cupule sessile on the axis, with a central point marking the vascular supply. Chalazal end of detached seed shows a large elliptical scar of attachment; micropylar end not preserved.

This genus too is very well represented at Onthea and is based upon a number of well preserved though incomplete specimens. The "flower" consists of a much elongated axis which functions as a female receptacle bearing numerous crowded orthotropous seeds all round it. There are no megasporophylls in the ordinary sense but the seeds are borne directly or almost directly upon the receptacle, with only a small intervening basal cup-like structure which itself is sessile on the axis. With the meagre data at hand it is impossible to say anything about the real affinities of the plant. At first sight a distant comparison with the Bennettitales may suggest itself, but there is no indication of any interseminal scales, nor has a perianth yet been discovered. For the present the systematic position of this extraordinary type of fructification must remain an open question.

Ontheostrobus sessilis sp. nov.

(Pl. XIII, Photos. 1–5; Pl. XIV, Photos. 6–9)

(Specimens 0/76–0/79, 0/81–0/90, 0/102; Also the big slab from Onthea)

Specific diagnosis

A much elongated receptacle bearing numerous sessile closely crowded seeds (broadest diam. about 6 mm.) all round through its length. Receptacle

about 3·5 cm. long (actual base and apex not preserved), about 1·6 cm. × 1 cm. in diameter at the base (? flattened by pressure), above which it rapidly narrows down to about 7 mm. × 4 mm. diam. and then tapers very gradually from below upwards to about 3 mm. diam. at the top. The seed is placed in a shallow cupule which itself is seated on a cushion; the cushion, in turn, is sessile on the axis. Where the seeds have fallen off a number of elliptical areas are left on the receptacle; these are the exposed surfaces of the cushions, which are themselves slightly hollowed out and show in the centre a small pit marking the vascular supply of the seed. The micropylar end of the seeds is never preserved. The detached seeds have a large elliptical scar (about 2 mm. × 3 mm.) at the chalazal end, evidently showing the area of attachment with the cupule.

Locality: Onthea in the Rajmahal Hills. *Collection*: Sahni and party (28th January 1942). *Horizon*: Rajmahal Series (Upper Gondwana).

The receptacle: mode of attachment of seeds

Out of about ten more or less complete specimens collected at Onthea, five show well preserved receptacles with some of the seeds still attached round them (Photos. 3, 6, 8, 9) while the remaining specimens only show groups of seeds detached from the axis but still packed into closely crowded groups and exposed by their chalazal ends (Photos. 5, 7).

Specimens No. 0/77 (Photos. 1–3) when split revealed the presence of a large number of seeds attached to the long tapering receptacle. The piece which came off first is shown in Photo. 1 and the receptacle is shown in Photo. 3. A similar number of seeds was expected to be attached on the other side of the receptacle and on careful splitting another piece came off which is shown in Photo. 2. Thus all the seeds represented in the Photos. 1, 2 were attached round the receptacle shown in Photo. 3.

It is very difficult to form an idea of the entire length of the receptacle because the actual base and apex are not preserved, but the portion exposed measures about 3·5 cm. long. At the base it is 1·6 cm. × 1 cm. In passing from below upwards, at first it rapidly narrows to about 7 mm. × 4 mm. and then tapers very gradually towards the apex (Photo. 3). This is the most complete specimen and it is regarded as the holotype. Here the size of the seeds and of the scars (measuring about 3 mm. × 2 mm. at the base and about 2 mm. × 1·5 mm. at the apex) left by them on the receptacle decreases towards the narrow end of the receptacle, hence the thicker end is taken as the proximal part and the thinner end as the distal. About 75 more or less complete seeds may be counted in this specimen and these were attached all round the receptacle in a length of about 3·5 cm. Thus the

total number of seeds on the complete receptacle must have been very considerable.

Axes of a similar type are preserved in some other specimens, *e.g.*, 0/76 (Photo. 9), 0/78 (Photo. 8), 0/79, 0/81 (Photo. 6), etc., but none is so complete as the one just described.

A few seeds still attached to the receptacle are well seen in specimen 0/81 shown in Photo. 6. On carefully observing this photograph it appears as if the seeds were enclosed in a shallow membranous saucer-like or cup-like structure. But the preservation is not good enough to show whether this membranous structure was a cupule-like organ distinct from the seed or whether it only represents the persistent basal part of the integument of the seed, the rest of which is not preserved.

Receptacles from which all seeds have fallen off are also present. These usually bear a number of characteristic circular or elliptical scars left by the seeds. One of these receptacles appears in Photo. 4. The elliptical scar is a raised cushion slightly hollowed out on its exposed surface, in which the seed is seated. In the centre of this depression a small pit can be made out (marked *p* in this photograph). These pits probably mark the vascular supply of the seeds. Photo. 5 shows a group of seeds which were once attached on this particular receptacle but which came off as a single piece while splitting the specimen. The seeds are all exposed by their chalazal ends, and show the characteristic elliptical scar of attachment, in the centre of which a distinct pit is sometimes seen marking the vascular supply.

Photo. 8 shows another specimen consisting of numerous seeds crowded together in their original grouping. In the upper part of the figure a small portion of the receptacle (*r*), is still left intact. The side of the receptacle facing the reader shows a number of scars left by the seeds which were broken off with the counterpart. Where the receptacle is not preserved, the seeds are exposed by their chalazal ends and some of these show clearly an elliptical scar, representing the surface of attachment with the receptacle.

The Structure of the Seeds

We have seen that the seeds are closely packed and no interseminal scales are present. The detached seeds too have an elliptical scar at the chalazal end (Photo. 7) corresponding to the elliptical scar on the receptacle and evidently showing the area of attachment. This area of attachment measures about 2 × 3 mm.; above this the seed gradually widens out to about 6 mm. diam. The shape and size of the entire seeds is difficult to tell because the micropylar end is never preserved.

Affinities

With only this much information in hand it is impossible to say anything definite regarding the affinities of this form. These features are not presented by any known gymnosperm fructifications. With the Cycadales no comparison is possible because the organ which bears the seeds cannot by any stretch of imagination be regarded as a sporophyll. It is evidently homologous with a floral axis. With the mention of a floral axis the Bennettitales come to mind, but here again there are difficulties, because there is no trace anywhere of interseminal scales. If subsequently a perianth is discovered at the base of the axis (which in our material is incomplete) a comparison with the Bennettitales may come within the range of possibility; but even then the absence of interseminal scales will remain an obstacle to our including this genus within the Bennettitales unless we are prepared to extend the present definition of that phylum. There is only one consideration which may still bring this genus within the fold of the Bennettitales and that is the perhaps rather remote possibility that in the younger stage of development interseminal scales of a delicate character were present, but that with the growth of the seeds they became crushed out of recognition. This again suggests that Onthea is a locality deserving of a closer attention by palæobotanists than it has so far received. It is possible that with further search younger stages and more complete specimens of *Ontheostrobus* may be discovered.

From the above consideration alone the extraordinary interest of this genus is self-evident.

It is possible that further research especially when more material is collected, may prove some sort of relationship between *Ontheostrobus sessilis* and *Rajmahalia paradoxa* Sahni and Rao, which is regarded by its authors (1935, p. 712), 'as an inverted funnel-like disc (possibly part of a deciduous androecium) fallen from the top of a Bennettitalean receptacle and bearing on its inner surface the impress of seeds and interseminal scales once pressed against it, but now no longer preserved'.

In view of the sessile nature of the seeds the fossils described here are given the specific name *Ontheostrobus sessilis* sp. nov. This is the only species of this genus, of which ten specimens are available.

Summary

The forms described under the name *Ontheostrobus sessilis* come, as the name indicates, from Onthea. Most of the specimens show well preserved elongated receptacles bearing throughout their length numerous crowded

seeds. The seeds are sessile and placed in shallow cupules which in turn are seated on cushions, the cushions being sessile on the receptacle. The receptacles from which the seeds have fallen off, show the exposed surfaces of the cushions slightly hollowed out. A small pit in the centre of this hollow marks the position of the vascular supply of the seeds. The detached seeds, usually found crowded together in their original groupings, show an elliptical scar at their chalazal end; this marks the area of attachment of the seeds with the receptacle. It is a noteworthy feature that not a single seed shows its micropylar end.

With the present data in hand, it is not possible to express any definite views regarding the systematic position of this peculiar and interesting fructification. A distant comparison with the Bennettitales is, however, suggested.

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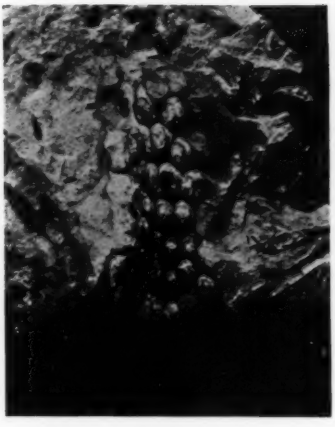
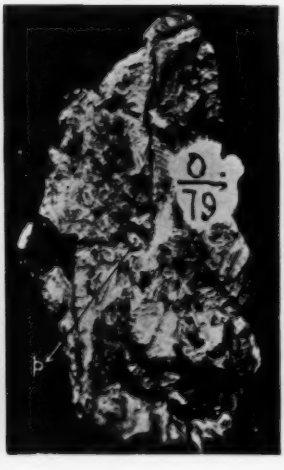


1.

2.

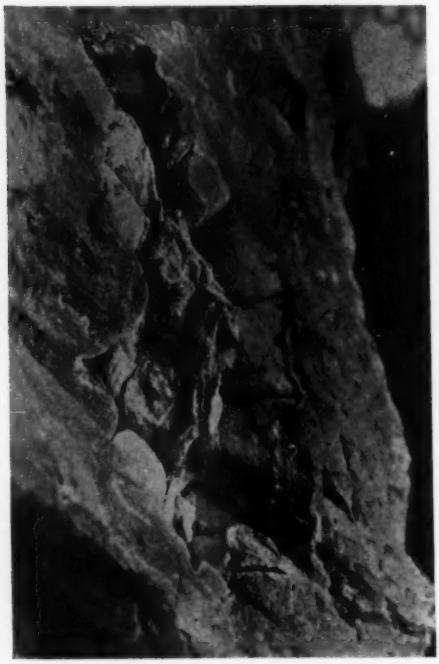
3.



4.

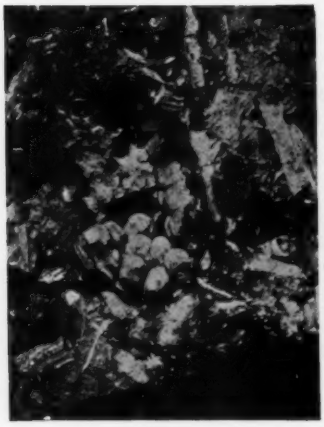
5.

FIGS. 1—5. *Ontheostrobus sessilis*



6. (×ca. 3½)

7. (×2)



8.

9. (ca. ×2¼)

FIGS. 6–9. *Ontheostrobus sessilis*

Explanation of Plates

All photographs are untouched. They are of natural size except where the magnification is given. The figured specimens are preserved in the Department of Botany and Geology, University of Lucknow.

Plate XIII

Ontheostrobus sessilis gen. et sp. nov. (Photos. 1–5).

Photos. 1, 2. Groups of seeds exposed by their chalazal ends. These seeds were attached all round the receptacle shown in Photo 3. 0/77.

Photo. 3. The receptacle showing a number of elliptical scars which were left by the seeds shown in Photos. 1, 2. 0/77.

Photo. 4. Portion of receptacle showing a number of elliptical scars. Some of the scars show distinctly a small pit (marked *p*) in the centre, probably marking the vascular supply of the seeds. 0/79.

Photo. 5. Counterpart of Photo. 4 showing some of the seeds which were attached to the receptacle shown in Photo 4. One or two seeds show the presence of a distinct pit in the centre. 0/79.

Plate XIV

Ontheostrobus sessilis gen. et sp. nov. (Photos. 6–9).

Photo. 6. Portion of receptacle showing a number of seeds still attached to it. It appears as if the seeds were enclosed in a shallow membranous cup-like structure. 0/81. × *Ca.* 3½.

Photo. 7. A group of seeds exposed by their chalazal ends showing clearly the elliptical scar of attachment. 0/78. × 2.

Photo. 8. Numerous seeds crowded together in their original grouping. In the upper part of this photograph a small portion of the receptacle (*r*) is still left intact and shows a number of scars left by the seeds. 0/78.

Photo. 9. Portion of receptacle with the attached seeds. 0/76. × *Ca.* 2½.

SYMPOSIUM ON STATISTICAL METHODS IN PLANT AND ANIMAL BREEDING

A SYMPOSIUM on the application of statistical methods to plant and animal breeding was held on 26th and 27th December 1946 under the joint auspices of the Indian and National Academies of Sciences during their annual session at Allahabad. The proceedings of the Symposium were initiated by Sir C. V. Raman on the opening day of the session by introducing to the audience the speakers who had assembled for taking part in the discussion. Dr. P. V. Sukhatme was in the chair. During the remaining sittings Mr. K. Ramiah took the chair.

Dr. V. G. Panse (Indore), opening the discussion, said that plant breeding formed one of the principal items of agricultural improvement everywhere. As an instance of the results achieved, he cited the sugarcane breeding at Coimbatore, pointing out that almost all sugarcane grown in India to-day belonged to one or the other of the large number of Coimbatore varieties that had been produced. Improved varieties in certain other crops like rice, wheat and cotton had also spread over considerable areas. While ordinary crops were susceptible to damage from pests and diseases, improved varieties showing a high degree of resistance to diseases like wilt in cotton or rust in wheat had been bred and their cultivation saved millions of rupees worth of crop from loss. Again, as in cotton, improved varieties had better commercial quality than types previously grown. The important point about plant breeding was that the improvement in crop was brought about without any extra labour or expenditure on the part of the cultivator who had merely to grow the seed recommended to him in place of ordinary bazaar seed. At the present juncture when they were anxious to grow more food in the country, cultivation of improved varieties would go a long way in making good the deficiency.

Until not long ago, plant breeding was considered to be an art. It was not necessary now to go into the merits of this view because this view had gradually altered and plant breeding was to-day recognised as much a science as, for example, agronomy or engineering was. This transformation had been brought about largely as a result of the application of statistical technique in plant breeding. Text-books on plant breeding acknowledged this role of statistics by providing extensive appendices on statistical methods; but the speaker wished to emphasise that the part that statistics should play

in the day to day work of the breeder was more fundamental and more essential than was ordinarily imagined. The breeder should find use for statistics, not only after he had accomplished the main task of evolving an improved strain, as was often supposed, but right through the whole process of breeding in order to direct his work along most effective lines. The speaker wished to present a brief review of the contribution that statistics would make to secure more rapid crop improvement.

The various problems in plant breeding could be divided into three groups. The first concerned the choice of material from which to breed. It was obvious that if improvement was to be effected in a crop there had to be scope for improvement ! In statistical language, improvement by breeding rested on the foundation of variability. Variability was of two kinds, genetic and environmental. In a field the individual plants differed in their performance with respect to any character in which the breeder might be interested partly on account of differences in their genetic constitution and partly due to differences in the conditions of their growth or environment. The variability observed in a crop, whether in a commercial field or of hybrid origin, was thus a combination of both kinds. The environmental component of variability was, however, of no use to the breeder or rather hindered his work. If plants were selected for their superior performance, but if this superiority was mostly due to their better environment, the performance of their progeny would not differ appreciably from that of the unselected plants. Only plants with superior genes in them gave progenies with high mean values. In terms of variability, it was the genetic fraction of observed variability that determined the capacity of a plant population to respond to selection. The breeder's choice of material for selection consequently depended on the amount of genetic variability it contained. It was, however, not possible to measure genetic variability directly, because in any observational data, the genetic and environmental constituents were bound together indissolubly. Statistical methods had been developed for estimating the relative proportion of genetic variability in plant material (Panse, V. G., 1940, *Journal of Genetics*, **40**, 283). Taking as an example, the improvement of wheat in U.P. by selection, the breeder might collect bulk samples of seed from several distinct tracts in the province and grow these in a replicated varietal trial. Their comparison under a common environment would bring out samples with high mean values which would indicate their genetic superiority. Further, if the character under selection was measured in a large number of randomly selected plants in each sample in the trial, a measure of gross variability present in the character would be obtained. Out of the plants observed, progenies could

be grown from as large a number of randomly taken plants as could be managed, in a replicated progeny row test. Then the regression of the progeny means on the values of parent plants gave an estimate of the genetic fraction of variability observed in the parent population. Such a study thus provided two criteria which could guide the initial choice of material. These were, mean values and genetic variability. Higher mean values assured a better mean performance in subsequent generations and greater genetic variability provided a larger scope for securing improvement over initial values. Where high mean value and large genetic variability went together the material was obviously the most suitable for selection. In samples where one of the criteria differed while the other remained substantially the same, that is, where variability differed but the means did not or *vice versa*, samples could be chosen on the basis of the variable criterion. But the problem presented a difficulty when both means and variability differed in opposite directions. A sample might have a low mean value but a high genetic variability or might have a high mean value coupled with low genetic variability. The question of preference between these two kinds of samples could not be decided on purely theoretical considerations. It was conceivable that even with a low mean value the variability might be so large that it could bring about, by a suitable degree of selection, improvement which would outstrip the improvement that could be attained by a similar degree of selection in a sample with a high mean value but low variability. The matter had to be examined experimentally. The speaker was studying at Indore the genetic variability in cotton samples collected from different parts of Central India and illustrated the point with results obtained in this study. Samples from 8 localities had been grown in a replicated varietal trial at Indore and about 150 randomly selected plants from all replications were examined for various characters, such as yield, earliness and quality in each variety. Replicated progenies from 30 random plants per variety were grown next season and the mean values of the progenies were correlated with parental values for estimating genetic variability. Results for fibre length which was the principal criterion for spinning quality in cotton, for three samples were given below.

Sample	Mean fibre length, m.m. in varietal trial	S. D. calculated from genetic component of variability
A	24·4	1·55
B	24·4	1·19
C	23·6	1·81

As far as samples A and B were concerned, sample A would be obviously the one to be preferred; the means of the two samples were very much the same but there was greater genetic variability in sample A than in B. The comparison of the third sample with these two presented a difficulty, because sample C had a definitely low mean value but a much higher genetic variability. Starting with the means of the samples and assuming a normal distribution with the standard deviation given above it was easy to predict the improvement that would result from the selection of a certain proportion of the best plants, by calculating the average for the corresponding portion of the tail of the distribution. For one per cent. selection, the calculated values were,

A	28·5
B	27·5
C	28·4

It was interesting to note that improvement in C, in spite of its much higher variability, just reached the level of improvement brought about in A which had the advantage of a higher mean value. This led to the conclusion that between mean and variability, the mean was the more important criterion in the choice of material. He had obtained similar results in other cotton material consisting of a larger number of crosses in the *arboreum* species and it appeared from all these results that in material usually handled by the breeder, genetic variability would be rarely so high as to more than compensate for the initially low mean value and hence material with higher mean value was to be preferred when the choice had to be made both on mean value and variability. These views were however based on the speaker's experience with cotton and a different situation might exist in some other crops. It was of utmost importance that breeders and statisticians should co-operate in examining other plant material in a similar manner in order to build up a body of information which would provide guiding principles in the choice of suitable breeding material.

If the conclusion that the choice should primarily rest on mean value was general in its scope, it led to some important and interesting consequences in relation to the stage at which selection should be commenced in hybrid material. Breeders started selection in such material only in the segregating generations such as F_2 or F_3 or frequently even later. If selection were to be based both on variability and mean it would be necessary to grow the F_2s of the crosses which were to be compared; but if mean values alone were to be taken into account, the comparison could be made at the F_1 stage and only such of the crosses which had the highest mean values could be retained for further selection within them. Breeders would

easily appreciate what immense saving of time, labour and expense this would mean, and these could be diverted to a more intensive examination in the subsequent generations of the few crosses chosen in the F_1. Immer had made a study of certain barley crosses through four generations and had reported that F_1 comparisons remained substantially unaltered in the subsequent generations (Immer, F. R., 1941, *Jour. Amer. Soc. Agro.*, **33**, 200). A question might be asked whether it was not possible to go a stage further back and compare the parent strains themselves for making crosses in which selection was to be made. The performance of F_1 could not be predicted accurately from the values of the parents concerned, because it could be shown theoretically that even parents with individually low values could produce hybrids excelling those from parents with superior values. In actual hybrid material extending over some 60 crosses in cotton (*arboreum* species), the speaker had found a correlation of 0·86 between F_1 and F_2 means but the correlation between F_2 mean and the average of the two parents was 0·75. Comparison of crosses by means of the F_1 therefore appeared to be a sounder procedure. As far as mean values were concerned selection in F_1 was as good as in F_2 on account of the strong correlation between the two.

The second group of the plant breeders' problems concerned the actual process of selection. In the early days breeders depended on what was termed ' mass selection '. The method was to collect the produce of suitable plants in the field, bulk the produce, raise the next year's crop from this produce, reselect suitable plants in this crop and grow them in bulk again. The process was repeated until a bulk with better performance than ordinary seed was obtained. The method of mass selection was replaced later by ' progeny-row-breeding ' which was found superior because mean values of progenies grown separately from the seed of single plants gave a more dependable criterion of their genetic potentiality than the observed values of individual plants. This could be demonstrated by a statistical reasoning.

Supposing that there was a group of plants belonging to n progenies each consisting of k plants, there would be a total of nk plants. The genetic component of variability in this group of plants might be designated as g and the environmental component by e. If the n progenies were grown separately and assuming no genetic variability within a progeny the analysis of variance of the results could be set down as

Due to	Degrees of freedom	Variance
Progenies ..	$n-1$	$kg+e$
Plants within progenies ..	$n(k-1)$	e

The variance between progeny means would be $g + e/k$ while that between individual plant values within progenies would be e. If the material were grown as a bulk of nk plants, not keeping the progenies distinct, the total variance would be $\frac{k(n-1)}{nk-1} g + e$. The coefficient of g was a fraction less than 1, except that this coefficient would be equal to 1 when k was equal to 1. Comparing the variances for progeny means and for the bulk it was clear that in progeny row breeding the genetic component formed the dominant fraction of variability whereas in mass selection the environmental component would be the larger fraction, except where the bulk was constituted by taking a single plant per progeny in which case the two fractions would be equal. In either case mass selection would be less effective than progeny row breeding, being subject to a greater influence of environment.

It should be noted, however, that even progeny means were affected by a fraction of environmental variability, e/k in the above analysis, and numerical differences between progeny means could not be used as a sufficiently reliable guide to genetic differences. A critical decision on this point could be made only by growing progenies in replication and using the standard error for comparison of observed differences in progeny means. It was in the matter of making replicated tests of progenies that considerable improvement was needed in the present-day methods. Various experimental designs particularly adapted to plant breeding material had been developed for this purpose and it was encouraging to find a growing realization of the value and utility of these designs on the part of plant-breeders. It was not necessary to enter into details concerning these designs here.

Though selection was now primarily based on progeny means, the desirable progenies had to be propagated from selected plants. This was the weakest point in the selection procedure because single plant values which were susceptible to a large degree of environmental variation had to be employed to represent the genetic constitution of superior progenies. With progenies grown in replication, single plants could be selected with greater confidence if the selection was based not on the observed value of a plant but on the deviation of this value from the mean of the plot. This would avoid undue weightage being given in selecting plants to those replicates which had given a higher performance owing to their more favourable location. Another approach to this problem was made possible by the discriminant function technique. In selecting plants for yield, for instance, the usual method was to select those plants that gave a higher yield. A second alternative was, to consider the components of yield and select with the help of a weighted linear function of these components, the weights depending on

the relative susceptibility of the individual components to environmental fluctuation and their mutual correlation. The yield of a wheat plant, for example, was the product of the number of ears, number of grains per ear and weight per grain. The yield of a cotton plant was similarly made up of the number of bolls per plant, number of seeds per boll and the weight of seed cotton per seed. The environmental and genetic variabilities of these components could be estimated by growing a number of varieties or lines in a replicated experiment and measuring the component characters in each plot. With these data the discriminant function technique enabled the evaluation of a formula in terms of the observed values of the component characters appropriately weighted and giving a numerical score most highly co-related with the genetic capacity of a variety or line. It was difficult to state in the absence of more experimental studies how much practical use the method had in helping single plant selection. The speaker's own conclusion from investigation on cotton and wheat at Indore was that when only components of the characters to be selected for were taken into account, the discriminant function did not appear much superior to straightforward selection on the character itself; but the discriminant function had the advantage in permitting selection to be based on characteristics which, though not components of the character to be selected, might be highly correlated with the latter. An example was provided by tillering in wheat which though not a direct component of yield was highly correlated with it and could be included in the discriminant formula thereby making selection for yield more efficient.

The third group of problems that the breeder had to tackle related to the maintenance of superior strains once these had been evolved. It was a common experience that such strains tended to deteriorate or run out in the course of time. Various causes were responsible for deterioration. One of them was mechanical admixture with inferior seed. In cotton, the seed was obtained from ginneries which were the principal source of mixture between varieties. Mixture might take place in the field also. Then there was cross-pollination with other varieties. A third factor responsible for deterioration was the residual genetic variability left in the strain. Although genetic variability was rapidly lost with the progress of selection a residue might persist when the strain was given out for commercial cultivation and this might lead to segregation of inferior genotypes and their spread in the strain. The breeder was aware of the necessity of maintaining the strictest possible purity in the nucleus seed, but he generally concentrated on morphological purity and in fact did not employ any means for testing the degree of purity in characters such as yield or quality for which the strain

was bred. The usual method of collecting selfed seed from typical looking plants for further propagation did not permit such a test being made. The proper method for this purpose was to maintain the strain by growing a replicated progeny row trial each season. In such a trial the possible beginning of deterioration could be detected at an early stage and suspected progenies could be discarded before bulking the rest for providing the nucleus seed. Elimination of inferior progenies would be made easier by making the test of significance less rigorous than the usual 5 per cent. probability level and it was to meet this need that tables of 'z' and the variance ratio for 10 per cent. level had been prepared (Panse, V. G., and Ayachit, G. R., 1944, *Ind. Jour. Agric. Sci.*, **14**, 244).

The speaker concluded his remarks with a reference to the need for a study of quantitative genetics. The earlier expectation that genetics would revolutionize plant breeding had not been borne out mainly because genetical investigation had been restricted to the formal mendelian characters while the study of quantitative characters which were the ones with which the breeder was really concerned had lagged far behind. This study could be pursued with the help of statistical methods coupled with adequate experimental material and provided a very rich field for co-operative work by Statisticians, Geneticists and breeders. The contribution that genetics had to offer to plant breeding depended on the advance in our knowledge of the behaviour of quantitative characters.

Dr. P. V. Sukhatme (Delhi), who led the discussion on the animal breeding side, said that a clear exposition of the rôle of statistical methods in plant breeding had been given by Dr. Panse. As the subject of symposium also included the rôle of statistics in animal breeding, he proposed to confine his remarks to the latter.

At the outset Dr. Sukhatme said that although an enormous change had been brought about in the plant-breeding methods as a result of the work of Dr. Panse and his colleagues at Indore, in animal breeding, they were a long way off from that ideal. The methods of animal breeding when contrasted with those of plant breeding were remarkably speculative and continued to be so in spite of the great progress in statistical science. This Dr. Sukhatme attributed to several factors. He said that whereas plants could be reproduced and multiplied many-fold every year, it took two to three years and even more to reproduce another generation of animals and five to six years for a group of animals to double their number. Then again whereas in agricultural crops the improvement could be effected by a few specialists from whom improved seeds could be bought and multiplied, the

improvement in animals rested with a large number of animal owners. Success in plant breeding had been attained by substituting the system of selection in which the value of an individual plant was judged by the average of its progeny for a system in which it was judged according to its individual performance. This, however, was not easy to accomplish in animal breeding. Then again an animal was relatively far more costly to experiment with than a single plant. For these reasons, he said, the methods in animal breeding stood relatively still in contrast to those adopted in plant breeding.

Despite these inherent limitations, Dr. Sukhatme said that statistical methods could play a valuable rôle in helping animal breeders to attain their aims. He said that he would illustrate this by describing a breeding project on goats financed by the Imperial Council of Agricultural Research.

The scheme was started with a foundation stock of 47 does and 4 bucks with the object of improving the average milk yield per day of lactation and of the kidding interval. It had been in progress for 10 years, when, he said, he was called upon to assess the progress made. He presented Table I

TABLE I

Comparison between the performance of the foundation stock and their first progeny

		No. of goats	No. of lactations	Total milk yield (oz.)	Length of lactation days	Average milk yield per day of lactation (oz.)	No. of goats	No. of lactations	Length of kidding interval days	Average milk yield per day of kidding interval (oz.)
Foundation stock	Average	19	68	4751·8	176·7	26·9	16	48	312·5	16·2
	Standard error			198·5	8·4	1·8			16·9	1·5
First progeny	Average	34	130	5832·5	176·1	33·1	30	110	291·4	20·9
	Standard error			210·3	5·2	1·4			9·5	0·8
t				3·3‡	0·1	2·9‡			1·1	3·5‡

showing the comparison between the performance of the foundation stock and that of its progeny and said that the progeny had given a significantly higher milk yield during per day of lactation and of kidding interval. This might not, however, be due wholly to the breeding policy followed on the farm. It might have resulted, for instance, from the better feeding and management right from the birth of the first progeny in contrast with the environment in which the dams were reared. Hybrid vigour might also partly offer an explanation. There might also be seasonal effect arising from the different climatic and disease conditions in the different years in which the dams and their daughters had their respective lactations.

He then presented a second table showing sire-wise comparison of the first progeny and their mothers and showed that the performance of the first progeny over their dams was significant in the case of sire No. 48 M alone and not in others. But even in the case of sire 48 M, he said, he could adduce evidence to show that the better performance of the first progeny was, to a very considerable extent, due to better feeding and management on the farm, to differences in the years in which the majority of the lactations of the foundation stock and the first progeny were completed and to the preponderance of winter kiddings in the first progeny. It was, however, difficult, to make any exact allowance for these factors. The best he could do was to compare the relative worths as transmitters of milk yield of bucks 48 M and 49 M as this comparison was free from consideration of the order of lactation or seasonal effects. The values of *t* of this comparison given in the last row of Table II indicated that while there was

TABLE II

Progeny tests for bucks 48 *M*, 49 *M and* 50 *M*

		No. of goats	No. of lactation	Total milk yield (oz.)	Length of lactation days	Average milk yield per day of lactation (oz.)	No. of goats	No. of lactations	Length of kidding interval day s	Average milk yield per day of kidding interval (oz.).
		(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)
48M Foundation stock	Average	11	38	4498·1	173·6	25·9	9	24	299·5	17·5
	Standard error			302·5	12·5	2·3			20·8	1·7
First progeny	Average	14	55	6199·5	178·2	34·8	12	45	286·9	23·2
	Standard error			353·8	8·1	4·4			15·2	0·8
t				3·4‡	0·3	2·8‡			0·5	3·2‡
49M Foundation stock	Average	6	25	4392·7	161·4	27·2	5	18	293·4	15·4
	Standard error			269·1	10·3	3·5			20·6	2·4
First progeny	Average	8	35	4920·7	157·0	31·3	7	31	268·5	18·2
	Standard error			346·8	7·6	3·0			15·2	2·0
t				1·1	0·3	0·9			1·0	1·0
50M Foundation stock	Average	1	3	5702·0	197·3	28·9	1	2	302·5	22·5
First progeny	Average	1	5	5891·2	167·4	35·2	1	4	337·5	17·0
t		Values of *t* for the comparison of the bucks 48M and 49M		1·8		0·7				0·8

a suggestion that buck No. 48 M was probably superior to 49 M, there was no adequate evidence to establish his superior worth over the other.

He next presented the results of the comparison of the second progeny with their mothers in the first progeny and those of the third progeny with their mothers in the second progeny and said that, far from showing any improvement, the results indicated a steady deterioration in the performance

TABLE III

Comparison between the performance of the second progeny and their dams

		No. of goats	Total milk yield (oz.)	No. of days in milk	Average milk yield per day (oz.)	No. of goats	Kidding interval	Average milk yield per day of kidding interval (oz.)
First lactation	1st progeny Average	18	5396·3	167·8	32·2	18	264·3	20·5
	Standard error		423·6	11·9	1·1		23·1	1·2
	2nd progeny Average	27	4864·1	176·9	27·5	25	313·7	14·8
	Standard error		349·7	11·9	0·9		23·7	1·2
t			0·9	1·7	3·2‡		1·4	3·6‡
Second lactation	1st progeny Average	15	5558·4	152·6	36·4	11	257·1	23·6
	Standard error		131·4	8·8	1·4		22·5	1·3
	2nd progeny Average	15	5216·0	192·0	27·2	12	0·0	15·7
	Standard error		102·5	9·8	1·4		3328·7	1·5
t			0·6	3·0‡	4·8‡		1·9	3·8‡
Third lactation	1st progeny Average	7	7906·3	206·0	38·4			
	Standard error		1416·6	28·2	2·1			
	2nd progeny Average	7	5617·4	215·7	26·0	Sufficient data not available		
	Standard error		931·3	29·6	2·2			
t			1·4	0·2	4·1‡			

TABLE IV

Comparison between the performance of the third progeny and their dams

	No. of goats	Total milk yield (oz.)	No. of days in milk	Average milk yield per day (oz.)	No. of goats	Kidding interval	Average milk yield per day of kidding interval (oz.)
2nd progeny Average ..	4	4703·5	148·3	31·7	4	256·5	18·4
Standard error ..		950·3	13·8	2·7		39·3	3·4
3rd Progeny Average ..	8	5260·9	227·3	23·2	7	392·3	13·4
Standard error ..		658·7	24·0	1·1		64·3	1·3
t ..		0·4	2·0	3·0*		1·4	1·5

TABLE V

Effect of selection in the foundation stock

Character compared		Foundation stock dams				First progeny daughters			
		No. of goats	No. of lactations	Value (oz.)	Standard error (oz.)	No. of goats	No. of lactations	Value (oz.)	Standard error (oz.)
(1)		(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)
Total milk yield oz.	First	9	35	5585·6	215·3	15	68	5683·7	286·5
	Rest	10	33	3867·6	265·1	19	62	5995·7	310·3
t ..				5·1‡				0·7	
Average milk yield per day of lactation oz.	First	9	38	30·3	2·3	16	63	32·8	1·9
	Rest	10	30	22·8	2·0	18	67	33·5	2·1
t ..				2·5*				0·2	
Average milk yield per day of kidding interval oz.	First	8	25	22·1	0·7	14	56	21·5	1·3
	Rest	8	23	12·2	0·9	16	54	20·4	1·0
t				7·9‡				0·7	

of both the second and the third progenies. He also presented the results of progeny tests for individual bucks giving similar conclusions. It was clear, he said, that while the first progeny showed better performance, there was a steady deterioration in the performance thereafter.

Dr. Sukhatme next considered the question whether selection from amongst the dams would have led to improvement in the successive progenies. An examination of this question was made possible because no culling of the female progeny was practised as a part of the policy followed on the farm. The whole of progeny was kept and bred up. For this purpose, he said, the mothers of the foundation stock were ranked in order of their performance and the results of the performance of the progeny of the first half were compared with those of the rest as in Table V. The results showed that while the performance of the second half of the foundation stock was significantly different from that of the first half, their progenies did not show a significant difference in the performance. He had similarly compared the results of the first and the second progeny and had reached identical conclusions, *vide* Table VI. It was clear, he said, that selective breeding on the side of the dams was without effect thus suggesting that there was no correlation between the performance of the dams and their daughters.

TABLE VI

Effect of selection in the first progeny

	Character compared		1st progeny dams		Standard error (oz.)	2nd progeny daughters		Standard error (oz.)
			No. of goats	Value (oz.)		No. of goats	Value (oz.)	
First lactation	Total Milk yield (oz.) ..	First	9	6861·6	380·9	17	4827·0	444·0
		Rest	9	3931·1	257·6	10	4927·2	636·8
	t ..			6·4‡			0·1	
	Average milk yield per day of lactation (oz.) ..	First	9	35·7	1·2	13	27·3	1·6
		Rest	9	28·5	0·9	14	27·7	1·0
	t ..			4·8‡			0·2	
	Average milk yield per day of kidding interval (oz.) ..	First	9	24·6	1·2	12	14·7	2·0
		Rest	9	16·9	0·5	13	15·0	1·3
	t ..			5·7‡			0·1	
Second lactation	Total milk yield (oz.) ..	First	7	6881·4	521·3	7	5092·7	411·1
		Rest	8	4398·8	180·1	8	5325·6	514·1
	t ..			4·8‡			1·1	
	Average milk yield per day of lactation (oz.) ..	First	7	40·7	1·7	7	24·2	1·6
		Rest	8	32·3	0·1	8	29·8	1·6
	t ..			4·9‡			2·5	
	Average milk yield per day of kidding interval (oz.)	First	5	26·7	0·9	6	16·5	2·4
		Rest	6	20·8	1·6	6	15·1	1·9
	t ..			12·7*			0·4	

TABLE VII

Coefficients of correlation between the performances of dams and daughters

	Total milk yield (oz.) (1)	Average milk yield per day of lactation (oz.) (2)	Average milk yield per day of kidding interval (oz.) (3)
Foundation stock and first progeny	0·170	−0·270	−0·012
First progeny and second progeny. First lactation	0·171	−0·005	0·064
First progeny and second progeny. Second lactation	−0·162	−0·397	−0·266

This, he said, was actually the case, as would be seen from the values of the coefficient of the correlation in the accompanying Table VII. These results

indicated that the foundation stock had a low genetic variability. He was rather puzzled to find that certain coefficients were negative, but, he said, they were not significant. It was possible, he added, that some of the assumptions underlying the estimation of genetic variability from the regression of the progeny means on the parental values were not realized in practice.

He concluded by saying that had the progeny tests been carried out in time, the results would have revealed a prepotency of the different sires and suggested suitable changes in regard to their use. As it was, he observed, four to five valuable years were wasted in trying to locate without success superior bucks by basing judgment on conformation instead of on the results of the progeny tests. Similarly a study of the genetic variability in the successive generations would have revealed the potentiality for improvement of the material and suggested suitable breeding plans. Yet another factor contributing to the lack of progress was the use of too few bucks to start with. He was glad that now, at any rate, the animal breeders had realized the potentiality of statistical tools as an help in attaining their objectives and were freely seeking the help of the Statistical Section of the Imperial Council of Agricultural Research in their work.

Mr. K. Kishen (Lucknow) said that it was the function of the plant-breeder to evolve out crop varieties giving high yield, of superior quality, resistant to disease, to lodging. For that purpose, the breeder had to experiment with a large number of plants or strains which he obtained by various methods of selections. Initially, the number of plants he had to select from was large. On account of the inadequacy of the seed material it was not possible to lay out randomised replicated trials at this stage. In making selection for the most promising varieties and the most promising progenies of a variety, the breeder had to take into consideration a number of quantitative characters for each plant, such as yield of grain per plant, ear number per plant, average number of grains per ear, and to select plants from the point of view of the requisite character or quality of the crop, *e.g.*, yielding capacity. Till the advent of statistical methods in plant breeding, this selection was essentially subjective and was attended with the risk that some promising strains or progenies might be rejected and undesirable plants or strains might be selected by this process. This selection was now best done by applying the method of the discriminant function.

Mr. Kishen then gave a brief outline of the method of the discriminant function and pointed out that selection by this method maximised the expectation of the genetic advance.

Further he observed that after the preliminary selection by the discriminant function the breeder was still left with a considerable number of varieties and progenies of those varieties. For the selection of the best varieties and progenies of those varieties, it was necessary to conduct replicated progeny row trials and lay out compact family-block designs. In those designs, with a large number of varieties, the size of block would become too large and require reduction in order that the elimination of fertility differences might be done efficiently. As a result of recent researches in the design of agricultural experiments, a large number of designs were available which brought about the desired reduction in the size of the block in such trials. Those were known as incomplete block designs, because, unlike the ordinary randomised block designs, the number of plots per block was less than the number of varieties, in these designs.

Let b be the number of blocks, k the number of plots per block, v the number of varieties, r the number of times a variety was replicated within a block and λ_{ij} the number of blocks in which ith and jth varieties occurred together. Such a general design was known to be analysable if it was a connected design.

He then briefly explained the concept of connectedness introduced by R. C. Bose, and cited the following two-dimensional square lattice in two equal groups of sets for 9 varieties as an example of an incomplete block design which was connected.

Block I.: 1 2 3	1 4 7: Block IV.
Block II.: 4 5 6	2 5 8: Block V.
Block III.: 7 8 9	3 6 9: Block VI.

He explained how in this case one could pass from any one variety to any other, say 1 to 9, by a chain of varieties and blocks like 1, I, 3, VI, 9 so that the design was connected. He said that it was interesting to note that a two-dimensional square lattice in one group of sets did not give a connected design.

If $V_1, V_2 \ldots V_v$ denoted the true varietal effects, then $\sum_{i=1}^{i=v} l_i v_i$, where $\sum_{i=1}^{i=v} l_i = 0$, was termed a varietal contrast. It was known from the theory of linear estimation that in a connected design every varietal contrast was estimable. Thus a connected design was analysable. He mentioned several examples of connected designs and added that the statistical theory of design of experiments had now advanced to such an extent that it was possible to provide the plant-breeder with an appropriate design which he

may require under any circumstances for laying out a suitable compact family-block trial and the parallel bulk trial.

He, further, observed that the number of varieties and the progenies selected by the above procedure would be small. It would then be necessary to conduct simple randomized block experiments with those varieties at a number of stations for about three consecutive seasons before the varieties which were most suitable for distribution among the cultivators were finally decided upon.

Referring to the statistical methods in animal breeding, he said that the technique of animal breeding was in its initial stages not essentially different from that of plant breeding. Selection by the method of the discriminant function was advantageous in animal breeding also. As an example he referred to a recent paper entitled "An application of the discriminant function for selection in poultry," by Dr. V. G. Panse (*Journal of Genetics*, 1946, **47**, 242) and pointed out that as a result of the use of the dicriminant function in this case, the percentage of genetic advance over straight selection varied from 10 to 30 per cent.

He remarked that animal nutrition experiments also formed a part of the science of animal breeding. In those experiments, the statistical principles of replication and randomization were extensively employed. In some cases, litters of animals, such as rats, had to be dealt with. In such cases, litters corresponded to blocks and animals within a litter to plots and the appropriate connected design, which in the simplest case might be a randomized block design, could be laid out for testing the efficacy of nutritional treatments.

He concluded by saying that it was sometimes necessary to test the efficacy on animals of several interacting groups of factors, *e.g.* dietary treatments. In such cases, it was appropriate to lay out a factorial design. But usually the main difficulty in laying out a factorial arrangement in those cases was the inadequacy of the number of animals available for experimentation. That difficulty had been largely overcome by the device of fractional replication of a factorial arrangement, introduced by D. J. Finney, and extended since by the speaker himself. He illustrated the theory of fractional replication by considering the 2^4 factorial design, where there were 16 treatment combinations, but where replication of half, *i.e.*, eight, of the combinations could yield valuable information regarding main effects and the more important interactions. Thus, factorial designs with fractional replication, in that they enabled factorial experiments to be conducted with one-half, one-third, etc., of the total number of treatment

combinations were likely to be very useful for exploratory experiments in animal nutrition.

Mr. V. D. Thawani (Delhi) said that he would deal with the discriminant function once more although Dr. Panse and Mr. Kishen had referred to it earlier, since it was a very useful instrument of plant selection. He pointed out that the equations from which Mr. Kishen had started were $X = \Sigma b_i x_i$ and $\phi = \Sigma a_i \eta_i$, where x_i and η_i were the phenotypic and genotypic values, and that a_i's were given and b_i's were chosen in such a way that correlation coefficient, ρ, between X and ϕ was the greatest. This could be looked upon from a different angle as well. The idea of plant selection was that the mean genetic value of the selected part should be as much as possible in excess of the mean of the unselected population. This excess might be called the genetic advance. If they had a large number of progenies and they had to select one-qth part of them for further propagation; they had to maximise $E(\phi) - \bar{\phi}$, where $E(\phi)$ was the expected value of the selecetd part and ϕ was the mean value of the whole unselected population. It could be shown that, that was equal to $\frac{z}{q}\sqrt{\text{Var. }\phi}.\rho$, where z was the ordinate marking off the one-qth part of the area of the standardised normal curve. Thus maximisation of ρ was the same thing as maximisation of $E(\phi) - \bar{\phi}$. Thus selection was based on the idea of the greatest genetic advance. It was understood that the one-qth part of the progenies which were selected for further propagation corresponded to the highest phenotypic values x.

His second point was that b's were expressed in terms of a's and certain variances and covariances, whose estimates were obtained from the analyses of variance and covariance. In that connection he asked them to consider in particular the equation $x_i = \eta_i + \xi_i$ where x_i, the phenotypic value, was made up of two parts η_i, the genotypic value and ξ_i, the component due to environment. There it was assumed that the two parts were additive, which gave

$$\text{Var}(X_i) = \text{Var}(\eta_i) + \text{Var}(\xi_i),$$

$$i.e., \; d_{ii} = e_{ii} + f_{ii} \text{ (say)},$$

if Covar $(\eta_i - \xi_i) = 0$ *i.e.*, the two parts η_i and ξ_i were assumed to be independent.

Then the analysis of variance gave the result:

Source of variation	Mean square
Between progenies	A
Within progenies	B

Obviously, B was an estimate of f_{ii} and A of d_{ii} and therefore A–B was an estimate of e_{ii} since $d_{ii} = e_{ii} + f_{ii}$. If A–B happened to be negative in practice, they took $e_{ii} = 0$. That led to a difficulty and it appeared advisable to look into the assumptions of additiveness and independence of genotypic and environmental components, on which the derivation of estimates of genotypic and phenotypic variances was based. He expressed doubt about the correctness of those assumptions and suggested that the operation law might be analogous to the vector law in place of the simple additive law.

Coming to the next point he asked them to consider the equation

$$X = \Sigma b_i x_i.$$

If one of the characters, say x_n, was unimportant and was dropped, a_n might be put equal to zero. In that case the ratios of a_1, a_2....changed thus changing the values of b's altogether. The recalculation of b's involved heavy computation and it might be possible to define functions of b's (say linear) which did not change even if any one of the characters was finally dropped. This point needed to be looked into mathematically.

Then he came to his last point and said that the discriminant function X was taken as a linear function of x_i's, *viz.*, $X = \Sigma b_i x_i$. He asked if it was possible that a non-linear function,

$$X = \Sigma b_i x_i + \Sigma b_{ij} x_i x_j + b_{ijk} x_i x_j x_k + \ldots.$$

might give a better result than a linear one, and if in such a case it was possible to define b's in such a way that they remained unaltered even when only the linear terms were taken.

Mr. S. D. Bokil (Indore) considered theoretically the relationship between hybrid vigour and F_2 and F_3 variances in the light of a simple Mendelian scheme of factors. He said that the following assumptions were made regarding the inheritance of quantitative characters, such as staple length in cotton:

(i) Such characters were affected by a large number of independent factors each having individually small effect.

(ii) The factors had no epistatic relationships.

(iii) They had equal effect on the particular character.

The justification for these assumptions was, firstly, that they were simplifying, and secondly that the assumption (i) was necessary to explain continuous variation of such characters while assumptions (ii) and (iii) gave results approximating to those obtained without them (Fisher, R. A., 1918, *Trans. Roy. Soc. Edinb.*, **52**, 399; Panse, V. G., 1940, *Journal of Genetics*,

40, 283). Under these circumstances the means and variances obeyed the additive law, *i.e.*, they were the algebraic sums of contributions derived from individual factors.

He then proceeded to consider the crossing of two pure varieties, *i.e.*, varieties homozygous for all factors concerned. Let one variety contribute p factors not contributed by the other favouring big size and other variety q similar factors. Only such factors needed to be considered since factors common to both the parents could not have any effect on hybrid vigour or variances of subsequent generations. It was obvious that the F_1 generation would be heterozygous for $p + q = n$, say, factors.

The three phases of any factor could be denoted by d, h and $-d$. If there was no dominance, $h = 0$ and the three phases would be denoted by d, 0 and $-d$. The expectation of the mean of the F_1 generation would be 0 in that case. On the other hand, if all factors were fully dominant for big size the sum $\sum_1^n h = nd$ or $= n$, if measurements be made in units of d, would represent F_1 value. If $\frac{2}{3}$rd of the factors were fully dominant for big size and $\frac{1}{3}$rd for small size the sum $\sum_1^n h$ and hence the mean of F_1, would be $0{\cdot}33\,n$.

The F_2 variance in the corresponding cases could be calculated by calculating the contribution of a single factor to F_2 variance. For each factor, $\frac{1}{4}$th of the progenies were homozygous for each of the two allelic phases, and $\frac{1}{2}$ heterozygous, the respective phenotypic values being d, $-d$ and h. The mean would be $\frac{h}{2}$, hence variance due to single factor would be

$$= \tfrac{1}{4}\left(d-\frac{h}{2}\right)^2 + \tfrac{1}{2}\left(h-\frac{h}{2}\right)^2 + \tfrac{1}{4}\left(d+\frac{h}{2}\right)^2$$
$$= \tfrac{1}{4}(2d^2 + h^2)$$

as had been shown by Fisher, Immer and Tedin (Fisher, R. A., Immer, F. R., and Tedin, Olof, 1932, *Genetics*, **17**, 107). Hence the F_2 variance due to n factors would be $\sum_1^n \frac{1}{4}(2d^2 + h^2) = \frac{1}{2}nd^2 = 0{\cdot}5n$,

for no dominance, *i.e.*, $h = o$. Similarly for complete dominance variance in $F_2 = \sum_1^n \frac{1}{4}(2d^2 + h^2) = \frac{3}{4}nd^2 = 0{\cdot}75n$

for both the subsequent cases, where dominance is either in one direction only or in both directions since $h^2 = d^2$ in both cases. He considered some

other cases and by similar methods calculated the mean variance within and between F_3 progenies also and presented the following table:—

Type of dominance	F_1 (Hybrid vigour)	V_{F_2}	V_{F_3} (between progenies)	Max. Genotype
No dominance ..	0	$\cdot 50n$	$\cdot 50n$	n
Full Dominance—				
(i) All factors for big size ..	n	$\cdot 75n$	$\cdot 56n$	n
(ii) ¾ for big, and ½ for small size ..	$\cdot 33n$	$\cdot 75n$	$\cdot 56n$	n
Partial Dominance—				
(i) $h = \cdot 5$ for all factors ..	$\cdot 5n$	$\cdot 56n$	$\cdot 52n$	n
(ii) $h = \cdot 8$ do ..	$\cdot 8n$	$\cdot 66n$	$\cdot 54n$	n

The mean variance within F_3 progenies was exactly half the variance in F_2 and could be calculated from the above table. All cases had the same maximum genotype indicating the same degree of maximum improvement attainable by selection. It was apparent from the table that with a given number of genes and with the same type of dominance, the variance in F_2 was higher for a larger value in F_1 and consequently even if variances as well as mean values were to be taken into account in selection, selection in F_1 would be reliable, as F_1's with higher values could also be expected to give larger variances in subsequent generations. If in actual experimental material this association between F_1 values and F_2 variances was not realized, a possible explanation was provided by the case for balanced dominance shown in the table. There the F_1 with a value lower than all other cases of dominance gave an F_2 with the largest F_2 variance; it had to be noted, however, that even here the F_2 variance was not larger than that obtained with full dominance for all factors which also had the highest F_1 value.

The large genetic component of F_3 variance between progenies as well as its approximate constancy in the different cases suggested that selection might be profitably exercised in F_3 generation; but, the expense of growing a large number of F_3 progenies would be considerable. The relative efficiency of selection in different generations was required to be studied carefully by weighting the considerations of expense and the improvement to be expected appropriately.

Mr. R. S. Koshal (Poona) said that there were three well-known methods of plant improvement, namely, importation and subsequent acclimatisation of foreign seed, mass or single line selection, and hybridization. He proceeded to describe the third method and to show how selection from suitable crosses could be made in F_1 generation without going further to the F_2 stage, thus saving considerable time and labour. Here the statistical methods had helped the plant-breeders to evolve a suitable technique

and the essential requirement of that technique is that the parents and the F_1's should all be grown the same year in a randomized replicated trial. Material for such a study was available from an experiment which had been carried out at the Institute of Plant Industry, Indore, and he illustrated the method with reference to three varieties and their F_1 crosses, which had been grown in the randomized blocks.

The varieties could be denoted by AA, BB, and CC and their F_1's by AB, AC and BC, where A, B and C represented the groups of genes in the parents responsible for fineness. There were 5 degrees of freedom for comparison among the parental varieties and their F_1s. These could be split up into 2 for the genetic part, 1 for heterosis and 2 for epistacy or interactions between genes. The genetic part gave comparisons between the three expressions $2AA + AB + AC$, $2BB + BC + AB$ and $2CC + CA + BC$, and these comparisons showed which variety was better for crossing. The comparison between the mean of the parents and their F_1's provided evidence for heterosis or hybrid vigour. The comparison was of the type:

$$AA + BB - 2AB,$$
$$AA + CC - 2AC,$$
$$BB + CC - 2BC.$$

The sum of these comparisons for the three crosses contributed a single degree of freedom for general heterosis. The remaining 2 degrees corresponding to epistacy consisted of comparisons between the quantities, $AA + 2BC$, $BB + 2AC$, and $CC + 2AB$. In the material under consideration it was found on the above analysis that the variety B which was Bani (*G. arboreum forma Indica*) was superior to the other two varieties, Malvi and Cwn. 520, for crossing. This illustrated how in some cases it was possible to eliminate undesirable material at F_1 stage if a suitable technique was employed.

He further stressed that as already pointed out by Dr. Panse, plant variability can be traced to two causes: (*a*) Environmental, (*b*) genetic, and it is the latter which plays an important rôle in plant selection work. For this purpose it is essential that plant-breeder should grow his material in replicated blocks and make selection on the basis of higher mean value and higher regression of progeny means on parental plants.

Mr. V. B. Sahasrabudhe (Indore) after referring to the importance of replicated progeny row trials emphasised by earlier speakers, said that standard layouts adopted for such trials consisted of randomised blocks with plots consisting of 5 to 10 plants replicated 5 to 10 times depending

upon the quantity of seed available. The method of growing progenies in strips with controls after 10 to 15 progeny rows was, however, adopted by some breeders. For a large number of progenies the various incomplete block designs would appear to provide suitable layouts.

He then discussed the results for two plot sizes 6′ × 2′ and 12′ × 2′ from a uniformity trial which was laid at the Institute of Plant Industry, Indore, with an Institute bred cotton strain, Dhar–43. The spacing between rows was two feet and between plants one foot, this being the standard spacing adopted for plant breeding trials at Indore. The results consisted of the following comparisons.

Comparison of the relative efficiency of simple randomised blocks with (1) strips with controls at regular intervals, (2) compact blocks with controls at regular intervals, (3) various incomplete block designs was shown in the following table:—

Relative efficiency of simple randomised blocks with strips and compact blocks with controls

Plot size	No. of progenies	Relative efficiency. (Efficiency of randomised blocks = 100)	
		strips	Compact block with controls
6′ × 2′	30	73·2	83·2
	40	72·8	83·8
	50	75·2	83·2
12′ × 2′	30	82·4	83·0
	40	77·7	87·5
	50	83·1	83·0

Both the designs were less efficient than simple randomised blocks by 12 to 27%. The reason for the lower efficiency of 'strips' was due partly to the undesirable shape of the blocks. Even compact blocks with controls were less efficient, due to the fact that controls occupied more space and had that space been utilised for increasing the number of replications the magnitude of the error variance could have been reduced more.

The relative efficiency of various incomplete block layouts for the 6′ × 2′ plot size without and with recovery of inter-block information, as compared to simple randomized blocks, was as follows:—

No. of progenies	Design	Relative efficiency (Efficiency of simple randomised blocks = 100)	
		Without recovery	With recovery
64	Double lattice ..	85·0	100·6
	Triple ,, ..	89·0	100·7
	Cubic ,, ..	66·6	100·0
	Symmetrical incomplete blocks ..	89·9	100·0
125	Cubic lattice ..	75·6	100·6
256	Double ,, ..	109·1	111·6
	Triple ,, ..	104·3	107·3
400	Double ,, ..	100·4	103·6
	Average ..	90·0	103·0

The results showed that for progenies upto 125 the designs were distinctly less efficient as compared to the simple randomized blocks, when the inter-block information was not recovered. For a larger number of progenies, however, the efficiency was slightly higher than of simple randomized block layout. With the recovery of information the efficiency was the same as that of the simple randomized blocks for progenies upto 125 and increased slightly for larger number of progenies. So far as the present results were concerned the designs did not seem to be useful considering the complicated nature of the field arrangement and the labour involved in the statistical analysis.

Mr. Sahasrabudhe added that in these designs the comparisons of the progeny means were done with unequal precisions as different errors had to be used for testing the significance of the differences when the progenies occurred in the same or different incomplete blocks, except in the case of symmetrical incomplete block designs, where due to the balanced nature of the design the progeny means could all be tested with equal precision. This latter design, however, required a certain minimum number of replicates, $p + 1$, if p progenies occurred in an incomplete block and could not be employed where the available seed was not sufficient for sowing the minimum number of replicates.

Dr. L. A. Ramdas (Poona) referred to the relationship between agricultural meteorology and plant breeding and explained how drought resistance of different varieties could be tested in the laboratory by controlling humidity and temperature.

Mr. R. D. Narain (Cawnpore) stated that there appeared to be a considerable similarity in the statistical approach to plant selection and to the

study of human abilities. He illustrated his remark by explaining how the discriminant function could be used in the evaluation of answer papers of examinations to assess intelligence.

Prof. S. Ranjan (Allahabad) felt that too much attention had been paid to plant yield in selection work in the past and too little to the nutritional value of improved varieties. He emphasised the need of physiological study in conjunction with plant breeding.

Dr. C. Chandrasekar (Calcutta) illustrated some applications of statistical methods to the study of human genetics. The science of human genetics presented greater difficulties than those presented by plant or animal genetics since neither ancestry and genotypes of human beings could be known with accuracy, nor could their matings be controlled. In such circumstances 'The principle of random mating' offered immense possibilities for the human geneticist. The principal assumptions of this method were that the matings took place at random, and that the genotypes were uniformly distributed in the population. With the help of this method several useful results could be calculated; for instance, the calculation of the proportions of normal and affected progenies in case of characters controlled by dominant or recessive genes. Similarly, it could be used to predict occurrence of certain characters and the results could be compared with those actually observed. One such character was the ability to taste phenyl-thio-urea in small dilutions. Expectations worked out for this character for American population were well corroborated by observed data. However, observational data on the occurrence of albinism in England differed very considerably from expectation and thus failed to substantiate the assumptions. That gene frequencies may vary substantially even in groups living close together was well brought out by the distribution of blood group data recorded at Calcutta. Hence it appeared exceedingly difficult to obtain actual results rigidly satisfying the condition of even distribution of genotypes. For the selection of homogeneous populations, primary surveys of blood groups, response to phenyl-thio-urea and such other characters might offer useful guidance.

Prof. Madhava (Delhi) said that he was not a plant or animal breeder but that his contacts with Drs. Sukhatme and Panse had aroused his interest in the subject and that he would say a few words as to how the statistician got involved in problems of that kind. It was true that the geneticist had to deal with phenomena due to a number of causes. He must, therefore, have resort to statistical methods. The statistician had also to enlarge the domain of his activities because statistics was a unifying element in all

sciences. However, he felt that there was too much emphasis placed on pure statistical methods. He had been reading in an amateurish way recently some of the books of Haldane and other authors. But there he noticed that his difficulty was not in following the general subject-matter. He came across certain equations of the kind:

$$\begin{aligned} Un &= Un + K/(Un + \overline{1 - K}) \\ &= (U^2n + Un)/(Un + \overline{1 - K}) \end{aligned}$$

He found that in Haldane's lectures there had been no formal solution of that equation. It had been solved in an approximate way only. The solution was valid where the generations did not overlap as in annual plants. He also had come across the transcendental difference equation in Fisher's book:

$$Un = Ke^{Un}$$

Pure mathematics had lagged and had not been able to devote itself to the rigors of a complete solution of equations of this type. Furthermore, one comes across certain types of linear equations of the 22nd degree in the evolution of pedigree proportions. Obviously, the solution of an algebraic equation of this order is impossible except through refined calculating machines.

Mr. V. G. Pendharkar (Bombay), in a written contribution, made the following observations on the Etah Goat Breeding Experiments in connection with the application of statistical methods to animal breeding.

Although the Goat Breeding Experiment carried on at Etah, to which Dr. Sukhatme had referred, failed in its chief objective, *viz.*, to breed a type of goats from the existing types with a higher milk-yielding capacity, considerable importance attached to the experiment on account of the revelation of the immense complexity of the problems which were inherent in such schemes.

A clear statement of the objective was the first necessity in any experimentation. The Etah Scheme aimed at producing goats with a higher milk yield. But the term higher milk yield could have several interpretations, such as a higher milk yield for the whole period of lactation, or a higher average milk yield for the period the animal is in milk, or again a higher average daily milk yield calculated on the basis of the interval between two kiddings. As the length of a lactation and therefore the length between two kiddings can to an extent be manipulated, it would appear desirable to aim at securing an increase in the average daily milk yield during the period the animal is in milk.

The selection of the raw material comes next. In order to have scope for improvement the raw material must possess a considerable amount of genetic variation in the values of the character with regard to which experiments are being made. The yield of milk, however, is a character notoriously susceptible to a variety of factors such as the time of the day, the stage to which a lactation has progressed, the order of the lactation, the type of feed given to the animal, the climate and so on. Appreciation of this fact implies (*a*) that in planning the experiment and during the analysis of the data due care has to be taken so as to prevent the mixing up of the effect of such factors and the effect of genetic selection and (*b*) that as a preliminary to breeding experiments a good deal of data should be collected and analysed so as to yield information regarding the relative importance to be attached to each of them. With a knowledge of the precise effects of the different extraneous factors on the character experimented upon we should be in a much favourable position for conducting the experiments. Besides, it is well known that the animal breeder attaches considerable importance to colour and configuration in fixing his " pure " types from the animals. Whether such " pure " types chosen so far in this country do in fact correspond to a " pure " type for a quantitative character such as milk yield or the yield of meat, etc., in the sense that the genetic part of variation in the incidence of these characters is negligible is a point which remains to be proved. In the Etah experiment most of the Barbari goats were chosen from a certain part of the country which is supposed to have a " pure " herd. The results of the experiment are not conclusive as to whether the goats and bucks were homozygous with regard to milk yield or not but there is considerable ground for supposing that this might have been the case. Prior knowledge particularly regarding the correlations between parents and offsprings would have been of immense help to the planners of the experiment had it been available. Collection and analysis of data regarding the incidence of such characters in the so-called " pure " types seems an urgent prerequisite for the animal breeding experiments in this country.

Secondly, information regarding the incidence of the character in one part of the raw material, namely, sires, is lacking. We can only estimate a sire's worth from the performance of his daughters which is too late for our purposes. Nothing can be more disheartening than to find after spending several years and considerable amount of money that (*a*) either the sires were probably homozygous with the dams and therefore there was no scope for improvement or (*b*) that they transmitted a low milk-yielding factor and actually spoilt the herd. Proper selection of the sires is therefore a very vital factor for the success of the experiment.

We must have a method of discriminating between good and bad sires and that too preferably at the start of the experiment or at any rate in its early stages, say, in the course of the first or second lactation of their progenies. This necessitates a very careful planning of the experiment and thorough analysis of the data by modern advanced statistical methods at each stage.

The necessity of such discrimination becomes all the more paramount on account of the practice of having a relatively small number of sires as compared to the dams. One of the main grounds of criticism against the Etah experiment is that the number of bucks was very small to start with and in the later stages the male progeny of only one of these bucks (48 M) was given great prominence. Since nothing was known regarding the ability of the original sires and dams and the first progeny for some unaccountable reason gave a higher milk yield than their dams the experimenters were lured into a false sense of security. By the time the true worth of the sires began to show itself the experiment was too far advanced. The lesson to be learnt is to have a relatively larger number of sires at the start of the experiment say one sire for every 15 dams or so. As far as possible the sires should be selected with reference to their mothers' lactation performances. All possible data regarding the performances of their relatives should be collected and studied. In the absence of herd books exact quantitative information would be impossible to obtain. And in any case the information will have to be supplemented by direct evidence obtained from the performance of their progenies. For this purpose the sires for the different groups should be interchanged for the different kiddings according to a pre-arranged plan so as to compare their abilities after allowing for the difference in the milk yield of the progeny caused by the variation in the dams. Even for such a scheme it would take 8–10 years before the true worth of the sires can be assessed. Though this may be so, the need for carrying out an experiment on the above lines can hardly be doubted. It may be possible to devise short cuts to forecast the sire's ability based on a study of material collected for a short period.

As mentioned above the first progeny of the goats on the Etah farm gave higher milk yield than their mothers. Various causes such as difference in the orders of lactations of mothers and daughters, difference due to the early nomadic life of the mothers, *i.e.*, the foundation stock and the farm life of the daughters, hybrid vigour, etc., have been adduced for this phenomenon. Whatever may be the actual cause, the phenomenon is worth investigating further because of the important issues it raises with regard to the breeding plan. Perhaps the easiest way of overcoming the difficulties created by such a phenomenon would be to regard the first progeny and

not the original animals as the foundation stock in the absence of information regarding the previous performances of the original animals. This would certainly mean some expenditure and loss of time but we would be on a surer ground in as much as we would be eliminating possible differences in milk yield caused by environmental factors. Secondly, we would have complete data for progenywise comparisons.

Associated with any such scheme must be an efficient system of recording and regularly analysing the data collected. Decisions regarding mating, culling, etc., should only be based on the results of the statistical analyses carried out by appropriate methods. We can then be assured of a reasonable chance of success.

Mr. A. R. Sen (Lucknow) in a written contribution made a survey of available statistical methods for the detection and estimation of linkage in genetics. He dealt with the method of maximum likelihood for estimation of linkage, alternative procedures when single factor segregations were disturbed and with the planning of efficient experiments for linkage studies.

Mr. K. Ramiah (Cuttack) in winding up the proceedings said that they had been listening to a series of important and thought-provoking papers on various aspects of statistics in its application to plant and animal breeding. He wished that many others who were actually engaged in plant breeding research were present at the meeting. The whole point of the discussion was that plant and animal breeders did not take enough help from statistics. As for himself he did not see any reason why the breeder should fight shy of statistics. After all, statistics was only a tool and it was necessary for the breeder to utilise this tool to make sure that he was proceeding on right lines.

He did not entirely subscribe to the accusation that breeders did not utilise statistics and said that statistics as a science in its application to agriculture and field experiments was hardly three decades old, and some of the problems dealt with by the previous speakers have been under investigation only within the last few years. He said he was one of those lucky few to be associated with men who were just bringing the idea of probable error and duplicate plots in agricultural experiments in India nearly thirty years back. Great improvements had taken place since then. He felt that in using statistics the availability of trained staff and other facilities had to be taken into account. Unfortunately, the statisticians were suggesting designs for the breeders without a correct idea of their requirements and the facilities available on the spot. The breeders were also, with a blind enthusiasm for the latest methods, trying to adopt them without understanding the

limitations and difficulties involved. He was glad to find from Mr. Sahasrabudhe's paper that a simple randomized blocks layout was really more efficient in several cases, than some of the later improvements like the incomplete block designs. While it was necessary to look for efficiency in a design, the practical convenience was, he considered, much more important particularly in out-of-the-way research centres with limited staff and inadequate facilities. Mr. Ramiah requested the statisticians particularly to look very carefully into the plant breeding problems before they gave advice. He wanted to warn the statisticians that they should not rest content with giving advice and examining the data collected but should also look into how the data had been collected.

He said that Indore had been unique in respect of an intimate association between the statistician and the breeder, but this advantage did not exist elsewhere in India. He was glad Dr. Panse had shown them how statistics could be of help in plant breeding and had drawn their attention to the value of the discriminant function in plant selection, but considering that very few statisticians had taken up the subject seriously, he considered that the progress made in India in the use of statistics in plant breeding was creditable. He hoped that Dr. Panse's results on cotton would soon be tested out in other crops and he was looking forward to their use in rice breeding. Referring to his recent visit to Denmark and Sweden, two of the most advanced countries in agriculture in Europe, he said he was surprised to see that the Fisherian technique was not adopted in field experiments in those countries. The workers there were content with the classical systematic arrangements in field trials on the plea that the application of randomization entailed practical difficulties and they thought that their method of balancing the varieties within each block gave a better scope for an easy visual comparison. The impression he got from a perusal of the recent Imperial Bureau publication on Soviet Genetics was that they were denouncing their faith in modern genetics, the basic science for plant and animal breeding, and were going back to Burbank's methods !

With regard to animal breeeding the position in India was very much worse than in plant breeding, as far as the use of statistical methods was concerned. This was entirely due to the fact that most of the animal breeders in the country were not acquainted with statistical methods; but there were signs of an awakening in recent years. Until recently there was not even unanimity of opinion among experts with regard to the parents to be used for crossing. Now that young men are being sent abroad for training in animal breeding and statistics he hoped that a better chapter would be written about animal breeding in the near future.

SPECIFICITY OF BACTERIAL SYMBIOSIS IN APHROPHORINÆ

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SCALE-INSECTS, when they contain symbiotic yeast-like micro-organisms, show specificity of symbiosis. The technique which gives such results is very simple. Smears of tissue containing the symbiotes are carefully illustrated with the help of a camera lucida and the drawings selected to show not merely representative forms but also to give a summary picture of the range of polymorphism observed. Such an illustration would represent all common polymorphic forms of the germ. If properly undertaken each picture would be different from others and exhibit specificity of symbiosis among insects as has been shown to be the case in some scale-insects.[1] This method was an extension of a plan[2] to classify commercial lac insects by a microbiological method and a subsequent study[3] has shown that lac insects also harbour specific symbiotes.

The method was extended and some interesting results were obtained. Morphologically the genuine lac insects and pseudolac insects were once classed in the same genus by an expert coccidologist like Green.[4] Taking three genera of lac insects, Lakshadia, Metatachardia and Tachardina, their symbiotes show a corresponding generic difference: Lakshadia have yeast-like symbiotes, Metatachardia contains an actinomyces,[5] while Tachardina species have bacteria. Now there is an insect, *Tachardina silvestrii*, which has not yet been recognised as an independent species by systematists. A smear from this insect easily distinguishes it from other sister species,[6] thus indicating the value of the new method when it can be applicable.

Tachardina lobata has two favourite host plants, *Michelia champaca.* and *Guazuma tomentosa.* Much to my surprise I found there are two separate insects[7] which do not differ morphologically but are separate with regard to host-selection and also with regard to the symbiotes they harbourr.

Just as pseudolac insects could be separated as a class from lac-insects proper, by generic differences in their respective symbiotes, some insects producing wax and pseudowax have been likewise separated. Cerococcus species secrete wax and they harbour yeast-like symbiotes, while a new genus

has been created, Coricoccus, for insects[8] that do not produce wax proper and contain bacteria in symbiosis.[9]

It has been previously emphasized that morphologists do not usually consider physiological characters.[2] If these be given due consideration specificity of symbiosis would also become a part of the physiological system of an organism which would be an additional aid in classifying the hosts exhibiting it. For example, it is being shown in this communication that two species of Aphrophorinæ differ in colour, a fact not fully exploited and further that their symbiotes also differ and it is these germs that produce their respective pigments. To admit that the colour of these insects differs from species to species is to imply that their producers, the symbiotic germs, also differ and independently this is being established in a series of studies which began with that of *Cicadella viridis*.[10] Specificity of symbiosis as far as yeast-like symbiotes are concerned, is relatively easy to establish; the method is not easily applicable to bacteria on account of their small size and further because the germs seem to disappear from the bacteritomes,[11] when the adults are about to lay the eggs. The method has more than one limitation but whenever the technique is applicable it is as reliable as any morphological method. When colour is recognised as an important character for differentiating species I do not see how the germs that produce these pigments can be overlooked. Since this point had not been recognised there appeared a great gap between the papers that established specificity of symbiosis where the symbiotes were yeasts and the series of communications that are being offered[10] to prove that there is a similar specificity of symbiosis even when the symbiotes are bacteria.

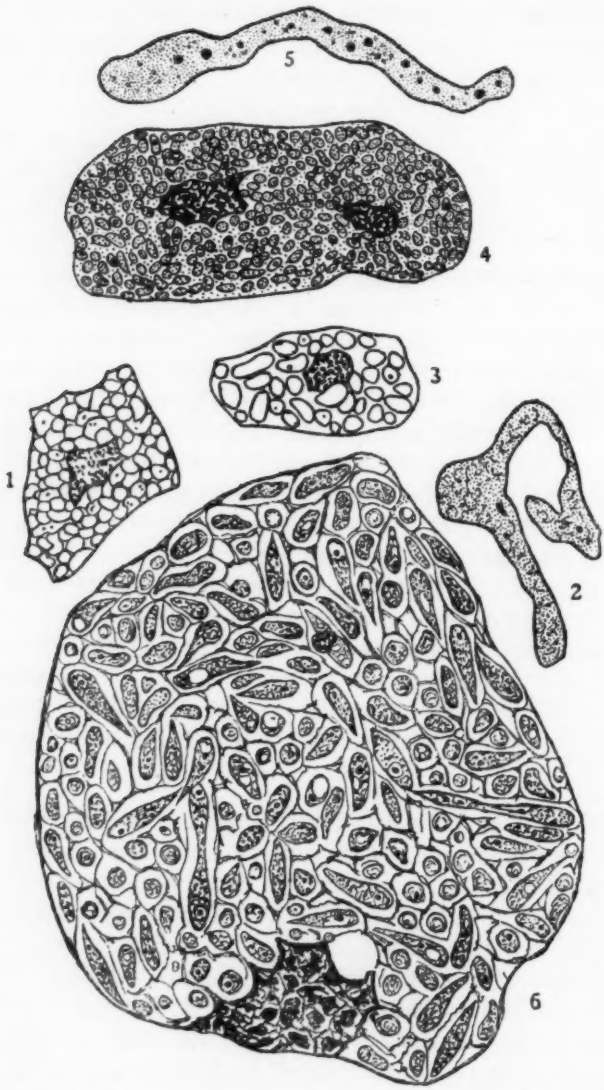
Every systematist recognises that it is easier to separate genera than to establish different species. The difficulty arises when morphological differences are small. It is precisely in such cases that physiological differences are greater. For example, *Tachardina silvestrii* and *T. lobata* have different host plants and different bacteria and also different colours. Dogmatic systematists have not recognised such facts as certainly Chamberlin[12] has not done, while my subsequent work has justified the new standpoint.

I shall begin with symbiosis in Aphrophorinæ of which only two species are commonly found in Europe. Haupt's monograph[13] of Homoptera gives *Aphrophora salicina* Goeze, also named *A. salicis* De G., as 9–11 mm. long and relatively less distributed. *Aphrophora alni* Fall. is 8–11 mm. long and is found all over Europe and even in north China. *A. spumaria* L.[14] is its another synonym and perhaps for this reason there appears to have been some confusion in identifying the insect. At any rate *A. alni* (*A. spumaria*)

is certainly the more easy to find and has consequently been the first species to be investigated. A popular book on natural history, like that of Lydekkar,[15] mentions only *A. spumaria* as a representative of the class of spittle insects. Sulc[16] records it in his classical communication on symbiosis, in 1910, stating, "Bei *A. alni* eine ganze Menge von eigenartigen grossen Bakterien, die im besonderen Zellen aufgespeichert neben der Hefe ganz gut in Organismus prosperiert" (p. 36). He thus records an yeast and a bacterium in *A. alni*.

In 1912 Buchner[17] published a monograph on symbiosis, incorporating studies on a species of Aphrophora. In the text he does not refer to Sulc's finding just mentioned. He illustrates a larva which bears no designation in the explanation to the plate where the coloured figure is given. Unfortunately I was led to state that Buchner did not know the species.[11] Buchner in his treatise, on p. 72, states that the species under question is *A. salicis*, the rarer insect. He however shows on Plate 11, Fig. 6 large bacteria-like cell-inclusions which, to quote Sulc, appear peculiarly large ones. The yeasts, to which Sulc refers, are also illustrated by Buchner on Plate 11, in Fig. 5. The illustrations fully confirm Sulc's previous record and added to this is the probability that Buchner also handled an insect which was by far the more common; the investigated *A. alni* and not *A. salicis* as he states. In his second monograph,[14] which appeared in 1925, Buchner further investigated the same insect where he confirms his previous findings of 1912, with the addition of a third yeast-like symbiote. Strange enough he does not correct the discrepancies to which attention has been drawn here.

A. salicis was actually first investigated by Buchner[14] in 1925. On p. 208 he classes the insect as a disymbiotic host. One symbiote is a long bacillus, typical of its class, to which no bacteriologist would object. He illustrates these germs in Fig. 4 *b*, on p. 103. The other symbiote is supposed to be a fungus or yeast, belonging to the mysterious genus Cicadomyces, and the species under question has been named by Sulc as *Cicadomyces aphrophoræ salicis* and quoted by Buchner[17] on p. 102 under the impression that he was studying *A. salicis*. It should have been *C. aphrophoræ alni*. A recent book on insect microbiology[19] by Sceinhaus also does not point out this mistake. The fact that so far such an obvious error has not been discovered is to be interpreted as due to the supposed germ having been very poorly described or, according to me, for not having existed at all. A tissue cell containing these *Cicadomyces aphrophoræ salicis*, shown by Buchner[14] in his Fig. 7, on p. 107, is reproduced here as Fig. 1. In a section



FIGS. 1–6. Fig. 1. Supposed symbiote of *A. salicis*, as an intercellular micro-organism from Buchner. Fig. 2. Supposed symbiote of *A. salicis*, isolated.—After Buchner. Fig. 3. Supposed symbiote of *A. alni*, as infection form, entering an egg.—from Buchner. Fig. 4. Supposed symbiote of *A. alni*, in a tumour cell of the adult insect.—from Buchner. Fig. 5. Supposed symbiote of *A. alni*, isolated, from the tumour of an adult insect.—from Buchner. Fig. 6. Picture of genuine yeast-like symbiotes, from a Cicad.—from Buchner.

these bodies do superficially resemble yeasts. But once they are isolated from the tumour these germs appear to have bizarre forms which have been also illustrated by Buchner in his Fig. 4*a*, p. 103. One of these bodies is reproduced here as Fig. 2. The object looks any thing but an yeast. These Cicadomyces have one appearance in sections and quite another when seen isolated. This is certainly nothing to do with polymorphism.

While discussing symbiosis in *Cicadella viridis*[10] I have mentioned that Cicadomyces are protoplasmic debris. In the tissue cell they lie in large pieces, such as Fig. 2 here illustrates, intertwined and folded so that none can be cut longitudinally. Imagine a ball of cord or thick rope being cut across; the cords in cross-section would always appear as round objects. Such is the case with Cicadomyces or elongated pieces of protoplasma which are always seen cut across and thus as round objects giving a uniform picture in practically all homopterous insects. The more these objects are studied the more is the uniformity discovered and it becomes impossible to give a specific description of any of these so-called germs. With Gimesa stain they take the characteristic blue plasma stain. They show no organised nucleus, but now and again only chromatin residues. Vacuoles are usually absent while they are always present in yeasts and in fungus mycelium. Yeasts and bacteria have some kind of membrane which enables them to withstand disintegration and even digestion with pepsin in dilute hydrochloric acid. The Cicadomyces do not possess any such membrane and are easily digested and disintegrated. None of them has been cultured so far while no microbiologist has seen anything like them anywhere outside the field investigated by workers on insect symbiosis. I must again quote from Gregson,[18] " During recent years much has been published regarding various intracellular symbiont like bodies. In several instances it has proved difficult to classify these bodies biologically and in a few cases it has not even been established that they are living units." This is perfectly true of Cicadomyces.[10] It therefore means that *A. salicis* contains only one symbiote, the bacillus first observed by Buchner.

In 1912 Buchner, confirming Sulc's findings, illustrated two symbiotes of *A. alni*, although he had mistaken it for *A. salicis*. Of these two symbiotes one was a giant form of bacterium, shown in his Fig. 6, Plate 11, and the other an yeast or rather *Cicadomyces aphrophoræ alni*, Sulc which was illustrated in Fig. 5, Plate 11. In 1925 Buchner reinvestigated *A. alni* now correctly identifying the insect. The previous findings naturally would have been of greater value if Buchner had also corrected the minor but obvious mistake of not having properly identified the species in 1912. The memoir

of 1925 records in all three symbiotes in *A. alni*, for which Buchner uses the synonym *A. spumaria* and under this name classes it as trisymbiotic insect, on p. 210. The three symbiotes are illustrated on p. 113 with their respective tumour-cells facing them, for easy comparison, on p. 112. The additional symbiote is another yeast-like object, evidently another Cicadomyces.

The largest symbiote in *A. alni* is Sulc's *Cicadomyces a. alni*. In Fig. 11*a* Buchner illustrates it as seen in a histological section where the tissue cells contain yeast-like or round objects ready to pass into an egg. One such tissue cell, full of Cicadomyces, is reproduced as Fig. 3 here. I am not sure if the author means to show two species of these mysterious germs or only one. However *Cicadomyces a. alni* is shown by Buchner in a section of the adult insect in his Fig. 9*a*, p. 112, which is copied here as Fig. 4. The supposed germ, when seen isolated, appears in Buchner's Fig. 10*a*, p. 113, with the same fantastic forms as were mentioned in connection with *A. salicis*. One of these forms representing *Cicadomyces a. alni*, shown isolated, is reproduced here as Fig. 5. *Cicadomyces a. alni*, Fig. 5, compares very well with *Cicadomyces a. salicis*, Fig. 2, so much so that neither in illustrations nor in living condition can there be any difference between them. Steinhaus,[19] who apparently believes in their existence, remarks in connection with *Cicadomyces a. alni* Sulc that "this species is without an adequate description". If a picture be drawn to show the polymorphism exhibited by the chief symbiote of *A. alni*, Fig. 5 here, with that of *A. salicis*, Fig. 2 here, there would hardly appear any difference. This is to be expected if both are protoplasmic debris. All the remarks passed previously with regard to Sulc's *Cicadomyces a. salicis* apply with equal force here.

In 1910 Sulc[16] mentioned two symbiotes, one is *Cicadomyces a. alni*, just dealt with and the second is supposed to be an exceptionally large bacterium. Buchner[17] illustrated these bacteria of Sulc in his thesis of 1912, in Fig. 6, Plate 11; and in his monograph[14] of 1925 on p. 113, in Fig. 10 *c*. These bodies are so large that no bacterium can be compared with them. Just as Cicadomyces occupy an anomalous position among yeasts, this bacterium of Sulc does among bacteria. Although Sulc did record finding a bacterium in *A. alni*, Buchner has not mentioned Sulc's finding, either in his work of 1912, or in that of 1925. His Fig. 10 *a* offered in 1925, remains unidentified either as bacterium or as fungus and my interpretation is that it is neither. Buchner merely calls it a symbiote.

Such long cell-inclusions, as these bacteria-like bodies appear, have not been met with in any other insect and as such struck to me as specific.

I could not have spent more time to study these bodies critically. They stain blue with Giemsa, as typical protoplasma pieces, are very delicate and do not stand disintegration, lack nucleus and many do not have vacuoles. I have done my best to cultivate them without any success. I am convinced they are not living entities but pathological products of protoplasmic origin. Objects of the same shape but shorter in length are met with in many insects.

The third symbiote which has been discovered by Buchner in 1925 and not mentioned in his thesis of 1912, is apparently a species of Cicadomyces, so that I am the more convinced that this new symbiote is only a stage of protoplasmic disintegration comparable with the main Cicadomyces. Not being different in kind to the other Cicadomyces, further criticism is not necessary.

To show what a picture genuine yeast-like symbiotes offer I have copied from Buchner[17] and reproduced his illustration, Fig. 4, Plate 6, as Fig. 6 here. They show yeasts in the fatty tissue of a Cicad; some yeast cells are seen budding which is hardly met with in sections showing Cicadomyces. The germs are separated from one another by residues of protoplasma from the tissue cell. Vacuoles are seen and likewise nuclei. Fig. 6 representing real germs offers a great contrast to Figs. 1 and 4 and 5 which all contain Cicadomyces or protoplasmic debris. My object has been to study the real symbiotes, to culture them and to see their role *in vitro*. The study of Cicadomyces has taken me to pathological histology which, I confess, has been rather forced upon me. I have also attacked this problem with respect to my predecessors who have been pioneers in the field, but we are all liable to error, and I have criticised them after much labour and patience in convincing myself.

Summary

Aphrophora alni and *A. salicis* each have one bacterium in symbiosis. In smears they are specifically different. *A. salicie.* has a long bacillus ; *A. alni*, a short and delicate bacterium. These germs produce the pigments of their host insects, the symbiote of *A. salicis* an ochre yellow pigment, like the colour of the insect, that of *A. alni* red-brown which is the colour of this species. Morphological and physiological tests have shown that the isolation of the symbiotes has been correct.

Sulc and Buchner have illustrated mysterious yeasts or fungi in symbiosis with these insects. These supposed germs are placed in a new genus, Cicadomyces. Details are given to show how they do not represent living entities. Even the authors themselves have subsequently discarded their earlier nomenclature and have designated these bodies simply as symbiotes,

feeling themselves doubtful regarding the real nature of the objects they have illustrated. These are best interpreted as protoplasmic debris or pathological products without any nucleus, but merely with chromatinous residues, without any membrane to resist disintegration and digestion and above all incapable of being cultivated and indicating any evidence with regard to their function. These Cicadomyces show a great contrast to real yeast-like symbiotes which have been also illustrated for comparison.

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ERRATA

Vol. XXV, Section B, No. 5, May 1947

Page 135, Table II, row No. 6, column 4:
The figure for total milk yield should be 4395·7 instead of 4392·7.

Page 136, Table III, row No. 2:
In the column under "Kidding interval" the figure should be 24·1 instead of 23·1.

Figure in the 8th row should be 330·0 instead of 0·0 and that in the 9th row should be 28·7 instead of 3328·7.

Page 138, Table VI:
The value of t in the last row should be 2·7 instead of 12·7.

ERRATA

Vol. XXV, Section B, No. 3, March 1947

Page 55, Para 1, line 7, "Bülbing" *should read* "Bülbring".

Para 2, line 10, "3 out of 55" *should read* "3 out of 5".

THE MODE OF ACTION OF NERVES ON UNSTRIATED MUSCLE

By Inderjit Singh, F.A.Sc., and Mrs. Sunita Inderjit Singh
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Received January 21, 1947

The views regarding the mode of action of nerves at their endings are well known. In the present research, an attempt has been made to determine the mode of action of nerves on unstriated muscle. Singh (1938 *a*) has shown that unstriated muscle shows two kinds of contractions. One kind is produced by alternating current and spontaneous activity (Singh, 1939), and the other by addition of substances to smooth muscle from without. If nerves act by producing a chemical substance outside the muscle fibres, then the resulting contraction would be of the second kind. Narayana and Singh (1944) found that in the dog's stomach, the calcium required for contractions produced by acetylcholine or by vagus stimulation, was more (0·005–0·01 *M* $CaCl_2$) than that required for the contraction produced by alternating current (0·002 *M* $CaCl_2$). The significance of this finding was not then realised; later on it was found to be a feature when the contraction belonged to the potassium group (Singh, 1945), thus suggesting that the contraction of the dog's stomach produced by stimulation of the vagus nerve was due to the secretion of a substance outside the muscle fibres, presumably acetylcholine.

Experimental

The muscle used in these experiments was from the stomach of the frog *Rana Tigrina*. Two kinds of muscle nerve preparations were made. In one, the entire stomach tied at the two ends, was suspended in a bath and the mesentery placed on a pair of electrodes; this recorded the contractions of longitudinal fibres. In the other, the two nerves supplying the anterior and posterior surfaces of the stomach were dissected down to a common segment which was then cut out transversely and then bisected longitudinally at the greater curvature; the mucous membrane was subsequently removed. This provides an ideal nerve-smooth muscle preparation for recording the contractions of the powerful circular muscle fibres. During stimulation of the nerves, the solution was lowered in the chamber, and the preparation suspended in the air. The nerves were stimulated by maximal induction shocks for 30 seconds every 15 or 20 minutes. As the responses of frog's

stomach are so variable, three pieces were taken from another or the same frog and the responses to alternating current, potassium and acetylcholine (I in 5000–2500) were obtained for comparison. This high concentration of acetylcholine was used as the frog's stomach is relatively insensitive, though responses obtained by nervous stimulation are usually powerful; inexcitability to nervous stimulation has not yet been found. During the same season the responses from the same batch of frogs are similar. The contractions by all kinds of stimulation are quite regular and can be obtained for several hours.

Results

The contractions produced by the longitudinal fibres are feeble, while those produced by the circular fibres are powerful when stimulated through the nerves; indeed the contractions are as powerful as those produced by any other form of stimulation. Spontaneous contractions may interfere, but often they are quite small. The latent period of the contraction produced by nervous stimulation is 5–20 seconds; adaptation may be rapid so that the response may begin to decline before the period of stimulation is over, or it may continue to increase for about 15 to 20 seconds after stoppage of the stimulus. The response is a twitch, but sometimes the relaxation is slow.

Nervous stimulation of circular fibres produces a contraction similar to that produced by alternating current and spontaneous activity while stimulation of longitudinal fibres produces a contraction which is similar to that produced by potassium and acetylcholine.

Action of Drugs

Effect of atropine.—1 in 10^{7}–10^{6} atropine sulphate inhibits the response to potassium, acetylcholine and of longitudinal muscle to nervous stimulation. 1 in 10^{5} then improves the response to nervous stimulation and potassium. The response to alternating current may be similarly affected, but usually 1 in 10^{7} improves the response to alternating current (Singh and Mrs. Singh, 1946), as well as the response to nervous stimulation of circular fibres. Higher concentrations are depressant to both. 1 in 10^{4} is depressant to all forms of stimulation (Fig. 1).

Acetylcholine action is sometimes very susceptible to the inhibitory action of atropine, 1 in 10^{7} and higher concentrations of atropine completely abolishing the response produced by 1 in 5000–2500 acetylcholine. At other times the muscle is very resistant, and the response persists even with 1 in 10^{4} atropine. When the response is a tonic contraction, then it is very

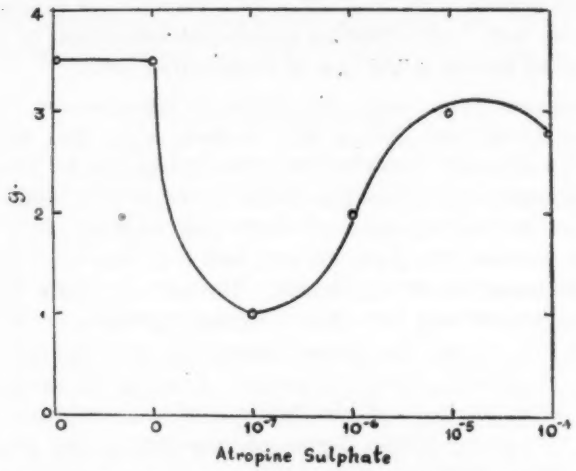

FIG. 1. Frog stomach; action of atropine on the response to nervous stimulation of longitudinal fibres.

susceptible; when it is a series of twitches, then it is more resistant. Calcium converts the tonic contractions of unstriated muscle into twitches (Singh, 1938 *b*). It would therefore appear that the membranes of muscles which respond by twitches contain more calcium and hence are less permeable than those which respond by tonic contraction. *Mytilus* muscles, which give twitches, swell less in various solutions, and so are less permeable than others (Singh, 1938 *c*; 1944 *a*). This is in agreement with Dale's view that the chemical transmitter in the case of vagal stimulation is secreted at a place at which atropine cannot penetrate. This is best explained if it is assumed that the chemical transmitter is liberated in the outer zone (Singh, 1944 *b*). The twitches produced by acetylcholine may be even increased by atropine (1 in 10^6).

Effect of eserine.—Previously it was found that small concentrations of eserine sulphate have an inhibitory effect on the response to acetylcholine (Singh, 1939). This was confirmed in the present series of investigations. It was found that tonic contraction is very susceptible to inhibition, 1 in 10^7 completely abolishing the response (Fig. 2). Thus with eserine also, tonic contraction is more susceptible than twitches. The response of longitudinal muscle to nervous stimulation is affected similarly; 1 in 10^7 causing inhibition, and 1 in 10^6 and 1 in 10^5, causing increase, the response being more resistant than that produced by acetylcholine. The response to alternating current and nervous stimulation of circular muscle is increased by 1 in

10^{-7}–10^{-6}, and depressed by 1 in 10^{-5}. The response to potassium may be affected either way. The response to nervous stimulation of circular fibres may be affected also as in the case of longitudinal fibres.

The response to nervous stimulation is characterised by long latent period, and by the fact that it may continue long after cessation of the stimulus. The question arises whether it is a composite or a single response. In one experiment only it was found that, to begin with, there was a single response; as the concentration of eserine was increased from 1 in 10^{-7} to 1 in 10^{-4}, the response was gradually split into two, one occurring during and the other on cessation of stimulation. The one occurring during stimulation was suppressed and the other occurring on cessation was augmented by 1 in 10^{-5}–10^{-4} eserine, the former resembling the response produced on stimulation of circular fibres by nerves. Thus nerves produce responses during and after cessation of stimulation, and these responses are affected differently by eserine. Other forms of stimulation also produce similar responses (Singh, 1938 *a*; 1939; 1942).

Effect of adrenaline.—1 in 10^{-7} improves the response to alternating current, and to nervous stimulation of circular fibres, or this action may be produced by smaller concentrations if 1 in 10^{-7} is inhibitory. Higher concentrations are depressant. 1 in 10^{-7} depresses the response to acetylcholine, potassium and nervous stimulation of longitudinal fibres. 1 in 10^{-6} has also a depressant action. 1 in 10^{-5} may potentiate the response to potassium as in *Mytilus* muscle (Singh, 1938 *a*) and nervous stimulation. Here again the tonic contraction by acetylcholine is more susceptible than twitches, 1 in 10^{-7} completely abolishing the response, and if 1 in 10^{-5} is depressant, then the response to nervous stimulation is more resistant, as with atropine and eserine.

Effect of acetylcholine.—The action of acetylcholine resembles that of eserine. It has two kinds of effects. First, it has an inhibitory action on the response to alternating current, potassium, acetylcholine and nervous stimulation of longitudinal fibres in small concentrations (1 in 10^{-6}–10^{-7}); in larger concentrations (1 in 10^{-5}), it has a potentiating effect. Secondly, it has a potentiating action on the response to alternating current and nervous stimulation of circular fibres in small concentrations, and an opposite action in larger ones.

The question arises, why, if the response to nervous stimulation is due to liberation of acetylcholine, the presence of acetylcholine in the saline then should not enhance the action of the former. The depressant action of acetylcholine is due to contracture or adaptation. In frog's stomach

contracture does not occur with small doses (1 in 10^5) as it is relatively insensitive, and adaptation to chemical stimulation is slow. It is thus found experimentally that 1 in 10^5 acetylcholine potentiates the response to nervous stimulation.

Action of Divalent Cations

Effect of calcium.—The optimum concentration of calcium for the response to alternating current is 0·0028–0·0042 *M* $CaCl_2$ and for acetylcholine, 0·0028 *M* $CaCl_2$; for potassium it is 0·0014 *M* $CaCl_2$ and for nervous stimulation, 0·0014–0·0028 M $CaCl_2$. In the absence of calcium, or if the concentration of calcium is reduced, there is a temporary state of hyper-excitability to potassium, acetylcholine and nervous stimulation.

Excess of calcium is depressant, but excitability again increases in about 0·007 *M* $CaCl_2$ as in avian and rabbit's gut muscle. The potentiating action of calcium to potassium as found in mammalian muscle is not found in frog's muscle, though this action is produced by excess of strontium.

Effect of strontium.—Strontium increases the response to all forms of stimulation in concentration of 0·0014–0·0028 *M* $SrCl_2$. The action of calcium on mammalian muscle is similar to that of strontium on frog's muscle. In frog's muscle strontium can replace calcium; as a matter of fact it may have a stronger effect. The stronger action of strontium appears to be due to diminished adaptation. This is probably due to diminished ionisation of calcium (Singh, 1944 *c*).

Effect of barium.—Barium has two kinds of action, one resembling that of calcium and the other that of potassium in causing contracture. Owing to its latter action, it is usually depressant. The calcium effect can be shown by the fact that it can produce persistent contracture in the absence of the Ca ion, and shows the calcium effect on the response to potassium, if contracture is not caused. The calcium effect is more evident in heart muscle; this is probably because the calcium in it is more mobile thus reducing its sensitivity to chemical stimulation (Singh, 1946).

Effect of magnesium.—Small concentrations of magnesium (0·0014 *M* $MgCl_2$) increase the excitability to alternating current nervous stimulation, potassium and acetylcholine. The response to potassium can withstand larger concentrations (0·0028 *M*), as in *Mytilus* muscle. The favourable action of magnesium is probably due to de-ionisation of calcium.

Action of Monovalent Cations

Effect of hydrogen ions.—The optimum pH for excitability depends on the buffer used. In borate it is about 9–8·5 and in phosphate, 8–7·5. In

phosphate the excitability remains unaffected while in borate the response to nervous stimulation is likely to fail. As the pH is decreased to pH 7, the response decreases and thereafter the response increases up to pH 6·5. If the pH is further decreased, the response to potassium, acetylcholine and nervous stimulation of longitudinal fibres increases up to pH 5·4–5·2 and the response to alternating current and nervous stimulation of circular fibres decreases. This is an important point in support of the view that response to nervous stimulation is due to the secretion of a chemical substance, and resembles the potentiating action of hydrogen ions on the response to potassium in mammalian muscle. Gessel and his associates (1944) have presented evidence that acetylcholine liberated by vagal stimulation may be potentiated by acids which retard the breakdown of acetylcholine by choline esterase. As acids also potentiate the response to potassium it appears that this potentiation is not due to an action on cholinestearase only.

Effect of lithium.—The antagonism between the response to nervous stimulation of longitudinal fibres and that to alternating current is well shown by the action of lithium (Fig. 3). Lithium up to 0·04 *M* LiCl decreases the response to nervous stimulation of longitudinal fibres, acetylcholine and potassium, the responses to alternating current being increased. With

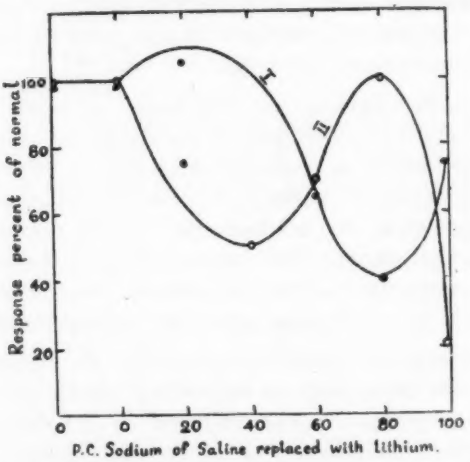

FIG. 3. Frog stomach. Action of lithium on the response to alternating current (Curve I) and nervous stimulation of longitudinal fibres (Curve 2).

further increase of concentration of lithium, 0·08 *M* LiCl, the response to the former three is increased and to the latter decreased. With complete replacement of sodium of the saline with lithium, the response to all forms

of stimulation is decreased, though that to alternating current may increases; the response to nervous stimulation of circular fibres is similar to that to alternating current.

Effect of sodium.—Replacement of 20% of the sodium chloride of the saline by sucrose decreases the response to alternating current and nervous stimulation of circular fibres and increases that to potassium, acetylcholine and nervous stimulation of longitudinal fibres. The action is however variable and may be just the opposite. In electrolyte-free medium (Singh, 1944 *d*), the response to nervous stimulation lasts for about an hour.

Effect of ammonium.—The replacement of about 60 to 80% of the sodium chloride of the saline with ammonium increases the response to acetylcholine and potassium but decreases that to alternating current, nervous stimulation of longitudinal as well as circular fibres. In these muscles, ammonium may cause contraction.

Effect of potassium.—The optimum concentration of potassium for the response to alternating current, nervous stimulation of circular fibres, acetylcholine and potassium is 0·01 *M* KCl; that for nervous stimulation of longitudinal fibres is one-half to one-third of the above. Potassium and ammonium are depressant to nerve. A higher concentration of potassium would be antagonistic to leakage of potassium from the fibres.

Action of Anions

Effect of bromide.—Low concentration, 0·02 *M*, may inhibit or increase the excitability; higher concentration, 0·04–0·06 *M*, has an inhibitory action. This action of bromide is probably related to its inhibitory action in the central nervous system. Higher concentrations, 0·08 *M*, potentiate the response to acetylcholine, potassium and nervous stimulation of longitudinal fibres; the response to alternating current and nervous stimulation of circular fibres is depressed. The replacement of all the chloride by bromide is depressant.

The action of nitrate, iodide and thiocyanate is similar, but is obtainable with smaller variable concentrations; they are much more depressant.

Effect of cyanide.—Small concentrations, 1 in 10^7, are inhibitory; 1 in 10^5 may then increase the excitability. 1 in 10^4 may increase the response to potassium and acetylcholine and depress others. The concentration of anions required to produce the above effects varies. 1 in 10^5 may potentiate the response to nervous stimulation of longitudinal of fibres, but usually cyanide depresses the response to nervous stimulation.

Effect of Osmotic Pressure

The effect of increasing the osmotic pressure of the saline is variable. Increase of osmotic pressure of the saline by adding sucrose to 1·4–2 times normal may increase the excitability to all forms of stimulation; at other times it may cause decrease. These variable results are probably due to the fact that increase in the concentration of potassium inside the fibres may antagonise either the excitatory or inhibitory action of ions outside the fibres; it will decrease the excitatory action of substances in the former case and increase in the latter.

Effect of Temperature

The optimum temperature for response to potassium and nervous stimulation of longitudinal fibres is 20° C. This suggests that in the case of latter stimulation, the stimulating ion is the potassium. The optimum temperature for nervous stimulation of circular fibres is 20–25° C., that for acetylcholine, 30° C., and for alternating current, 20–25° C. The optimum temperature may vary with that of the saline, thus exhibiting adaptation (Fig. 4).

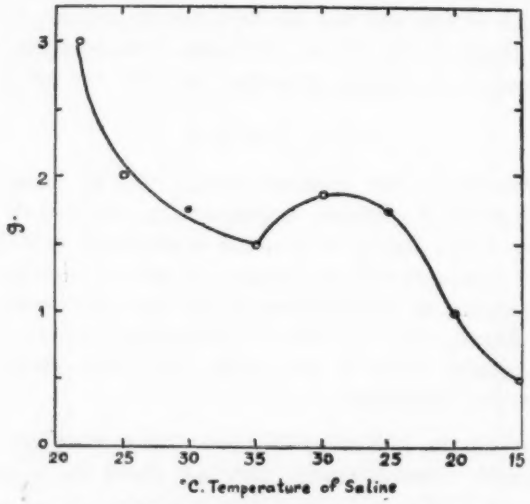

FIG. 4. Frog stomach. Effect of temperature on the response to nervous stimulation of circular fibres; note adaptation.

DISCUSSION

Dale and Feldberg have shown that acetylcholine is liberated from the stomach during vagal stimulation. This liberation of acetylcholine is of

functional importance as the contraction produced by vagal stimulation is similar to that produced by acetylcholine (Narayana and Singh, 1944). During nervous stimulation of frog's muscle the contraction produced resembles that produced by potassium or acetylcholine. It thus appears that acetylcholine is liberated, though the stimulating ion may be potassium. The potassium probably comes from within the cells, as increase in potassium concentration in the saline does not augment the response; acetylcholine probably causes depolarisation of the membrane, increasing its permeability, and causing leakage of potassium ions from within the fibres. These then cause stimulation as has been explained in connection with the occurrence of contraction caused by alternating current.

The action of atropine suggests that acetylcholine is liberated not around the fibres, but in the adjacent zone (Singh, 1944 *b*).

The occurrence of a contraction which is similar to that produced by alternating current, when nerves are stimulated, suggests the possibility of electrical transmission, which will precede chemical transmission, as found by Lorent de No in the central nervous system (McDowell, 1944). The function of chemical transmission would be to impart tonic properties to the phasic contraction produced by electrical transmission. The function of cholinestearase in certain situations may be to prevent this action where it is not desired; eserine by inhibiting its action would bring out the tonic function. It is possible that in some places in the body, the transmission is electrical, in others only chemical or electro-chemical; one or the other being suppressed as required.

Denervated structures become more sensitive to neurohormones. This suggests that these neurohormones are continuously or very frequently secreted. In unstriated muscle increased sensitivity to an ion follows when the muscle is deprived of that particular ion, such as calcium or potassium (Singh, 1942; 1946).

Summary and Conclusions

1. The nature of response of frog's stomach muscle to nervous stimulation is described. The contraction is similar to that produced by acetylcholine and potassium, and is not of the same type as that produced by alternating current, suggesting that acetylcholine is liberated during nervous stimulation of frog's stomach. Excitation by nervous stimulation appears to involve the potassium ion.

2. Nervous stimulation also produces a contraction similar to that produced by alternating current, thus suggesting that electrical transmission

precedes chemical. It is suggested that chemical transmission imparts tonic properties to the effects of electrical transmission.

3. On nervous stimulation, circular fibres of the stomach give the second kind of contraction, and longitudinal the first kind or tonic contraction. It is probable that the function of the longitudinal fibres is to maintain a tonic pressure on its contents and prevent the sagging of the stomach, and that of the circular fibres is to mix the contents by rhythmic contractions, as well as to exert a tonic pressure.

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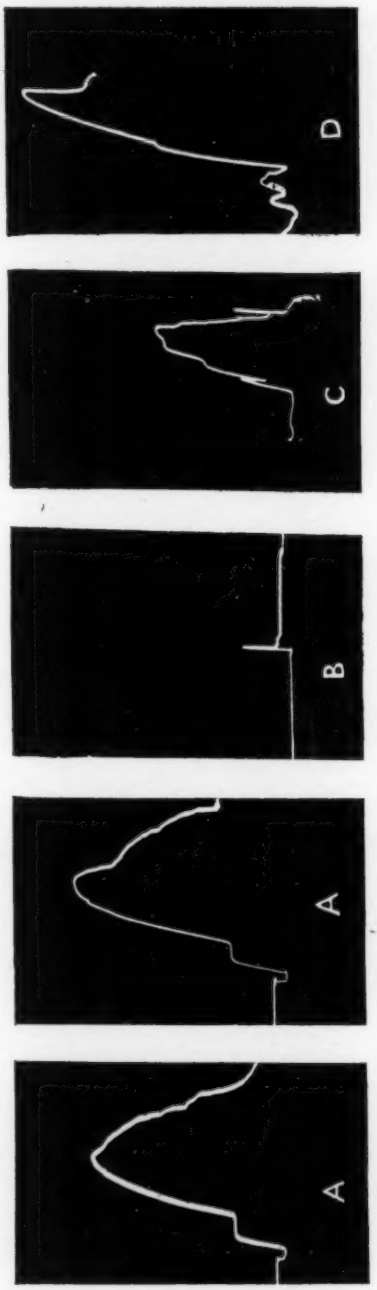

FIG. 2. Frog stomach. Action of eserine sulphate on the responese to acetylcholine (1 in 5000). A is normal response, B in 1 in 10^7, C in 1 in 10^6, D in 1 in 10^5.

INFLUENCE OF ROOT EXCRETIONS AND GERMINATING SEEDS ON NITROGEN-FIXATION BY AZOTOBACTER

By B. N. Uppal, F.A.Sc., J. A. Daji and M. K. Patel

(College of Agriculture, Poona)

Received March 24, 1947

In a previous paper (Uppal *et al.*, 1939), it was shown that *Azotobacter* from cultivated rice soils was stimulated to greater activity in fixing atmospheric nitrogen when grown in pure culture in association with growing roots of rice plants. This increased activity on the part of the micro-organism was further shown to be due to a change in reaction of the culture medium brought about by rice seedlings growing in it. In the experiments to be described below, the influence of the presence of growing roots of wheat, *jowar* (*Sorghum vulgare* L.) and radish on the fixation of nitrogen by *Azotobacter* in Ashby's cultures, has been investigated with a view to determining whether the living roots of these plants also stimulate the development of the micro-organism and thus enhance its nitrogen-fixing efficiency.

Experimental Methods and Results

For the purpose of inoculation, isolate III of *Azotobacter*, which has previously been shown to be an efficient nitrogen-fixer (Uppal *et al.*, 1939), was grown on Ashby's agar, and scrapings from a two days' growth of this micro-organism were used to inoculate the culture bottles containing 50 ml. of Ashby's solution.

Seeds from which experimental seedlings originated, were disinfected in a one in 500 solution of corrosive sublimate for 15 minutes. These seeds were then washed in sterile water and aseptically removed to sterilised glass chambers lined inside with sterile, damp filter-paper. When the seedlings had made growths of 2 to 3 inches in length, 9 seedlings of each kind were transferred to culture bottles.

Four sets of culture bottles each containing 50 ml. of Ashby's solution were prepared for each kind of plant and were treated in the following manner:—

(1) Inoculated with Is. III,

(2) Nine seedlings of each kind were grown in each culture bottle in this set,

(3) Same as in (2) above but each culture bottle was inoculated with Is. III, and

(4) Same as in (3) above, and in addition one gram of sterilised soil was added to each culture bottle.

At the conclusion of the experiment after 20 days, the total nitrogen was determined from duplicate samples of each culture bottle including the seedlings and soil. The results are given in Table I.

TABLE I

Influence of growing plant roots on nitrogen-fixation by Azotobacter

(Total nitrogen in mg.)

Ashby's solution containing	Radish		Rice		Wheat		Jowar	
	Individual bottle	Average	Individual bottle	Average	Individual bottle	Average	Individual bottle	Average
9 seedlings ..	4·31	4·27	1·66	1·51	7·71	8·03	4·20	3·93
	4·23		1·35		8·36		3·66	
9 seedlings+ ..	6·89	6·37	2·27	2·35	8·71	8·48	4·39	4·34
Is. III ..	5·85		2·44		8·26		4·30	
9 seedlings+ ..	9·43	9·02	5·06	4·88	13·23	13·44	7·23	7·31
Is. III+soil ..	8·61		4·70		13·66		7·39	
Net nitrogen fixed by Is. III in presence of seedlings and soil	3·17		1·79		3·83		1·80	

N.B.—Ashby's solution and soil in these experiments contained 0·06 mg. N and 1·58 mg. N, respectively. Is. III alone fixed 0·48 mg. N.

Results in the above table show that, as reported earlier (Uppal *et al.*, 1939), *Azotobacter*, in association with living roots of seedlings alone or in combination with soil, was able to fix larger amounts of nitrogen than in the absence of such association. The organism was aroused to greatest activity when wheat seedlings were used, and fixed 3·83 mg. {13·44—(8·03—1·58)} nitrogen, followed closely by radish (3·17 mg. N). Rice and *jowar* were poor in this respect and exerted an almost equal degree of stimulation. It may be noted, however, that, when wheat seedlings were used alone without the soil, the organism did not fix, in such association, as much nitrogen as when radish was used. The significance of these results is not quite clear at present.

Hiltner, as quoted by Waksman (1931), found that " non-symbiotic nitrogen-fixation is stimulated by growing plant roots; the higher plants use up the available nitrogen in the soil and thus create a nitrogen-hunger

for the non-symbiotic nitrogen-fixing bacteria. The plants supply the bacteria with available energy, in the form of rotting root, hairs, root tips, etc." Waksman and Starkey (1931) came to a similar conclusion—" in the neighbourhood of growing roots of plants there is an excretion of soluble carbohydrates and addition of other residues to the soil which may serve as food for bacteria. Plants rapidly consume most of the available combined nitrogen from this portion of the soil. These two factors, namely, the presence of available sources of energy and a nitrogen minimum, would favour the rapid development of *Azotobacter* and *Clostridium* and lead to nitrogen fixation ". Vyas (1934), working with maize seedlings, also noted that the maize roots excreted some stimulative product which enabled the non-symbiotic nitrogen-fixing micro-organisms to fix larger amounts of nitrogen. Viswa Nath reported (1939) that the gain in the nitrogen-content of the soil under field conditions may be partly due to the stimulating action of root excretions on the nitrogen-fixing bacteria.

Whatever may be the contributing factors, it is obvious that the association of growing roots of plants with *Azotobacter* stimulates the development of the latter and leads to an enhanced activity on its part in fixing atmospheric nitrogen, although it may be noted that the beneficial effect so exerted on the micro-organism varies with the type of associated plant. It is claimed, however, by some workers that sprouting seeds and living plants themselves have the power of fixing atmospheric nitrogen during germination and the subsequent growth of the seedling. Lipman and Taylor (1924) found that wheat and barley plants grown in culture solutions fixed nitrogen, but it may be noted that no attempt was made in these experiments to maintain sterile conditions and that tap water was used for making culture solutions. Burk (1937), in controlled experiments with peas, did not obtain any evidence of nitrogen-fixation during germination of pea seeds.

Sen (1929) has suggested the possibility that nitrogen-fixing micro-organisms may live symbiotically in the roots of rice plants, whilst Viswa Nath (1932) holds the view that the rice plant itself has the power of fixing atmospheric nitrogen. The latter (1940) has also reported that maize seed, when germinated in a known volume of air devoid of all combined nitrogen, absorbed atmospheric nitrogen during germination of the seed and the subsequent growth of the seedlings. Jamieson, as quoted by Winters (1924), claimed that all green plants possess the power of fixing nitrogen. On the other hand, Krassilnikov, as quoted by Lochhead (1940), has shown that *Azotobacter* was unable to grow in the rhizosphere of wheat, *i.e.*, the subterranean part of the plant system, and was severely suppressed in that of maize. He attributed this to the toxic effect of root secretions.

In view of the conflicting evidence on the ability of growing plants to fix atmospheric nitrogen, an experiment was done to determine whether seeds absorbed nitrogen during germination and the subsequent growth of seedlings. Seeds of wheat, *jowar* (*Sorghum vulgare* L.) radish and pea, which were previously disinfected in a one in 500 solution of corrosive sublimate for 15 minutes, were germinated on damp filter-paper in moist, sterilised chambers at room temperature. Fifty seeds and an equal number of 4- and 20-day old seedlings of each kind of plant were analysed for total nitrogen. Results are presented in Table II and show that germinating seeds and young seedlings do not possess the power of fixing elemental nitrogen when tested under aseptic conditions.

TABLE II

Nitrogen-fixation by germinating seeds and seedlings

(Total Nitrogen in gm.)

Kind of plant	Seeds		4-day old seedlings		20-day old seedlings	
	50 seeds	Average	50 seedlings	Average	50 seedlings	Average
Radish ..	0·026	0·025	0·023	0·025	0·029	0·028
	0·024		0·027		0·027	
Rice ..	0·007	0·007	0·008	0·008	0·007	0·007
	0·007		0·008		0·007	
Wheat ..	0·049	0·051	0·048	0·048	0·044	0·040
	0·053		0·048		0·036	
Jowar ..	0·020	0·018	0·020	0·019	0·019	0·018
	0·017		0·018		0·016	
Pea* ..	0·197	0·185	0·184	0·184	0·187	0·182
	0·174		0·185		0·177	

* In the case of peas, 25 seeds or seedlings were used.

SUMMARY

In the presence of growing roots of wheat, radish, rice and *jowar* in Ashby's cultures, *Azotobacter* fixed larger amounts of atmospheric nitrogen than in their absence. The stimulating effect, however, varied with the kind of plant used, wheat exerting the greatest beneficial effect followed closely by radish. Rice and *jowar* were poor in this respect and exerted an almost equal degree of stimulation.

None of the seeds tested possessed any power of fixing elemental nitrogen during germination and the subsequent growth of the seedlings.

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ADDITIONS TO FUNGI OF MADRAS—II*

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(4) *Puccinia solani-giganteæ* sp. nov.

This rust is found on the leaves of *Solanum giganteum* Jacq. at Naduvattam, Nilgiris. Its presence can be recognized by the groups of orange-yellow sori in the midst of the thick white tomentum on the lower surface of the leaves. Corresponding to these, small rusty brown spots are seen on the upper surface of the leaves.

Pycnia are developed towards the upper surface. They are subepidermal globose, 100–180 μ, and yellowish-brown in colour. Paraphyses and spores are present in the pycnia.

Aecia are hypophyllous, cup-like, formed in groups one to three millimetres in diameter, each cluster having a variable number of sori. The æcium is sunk in the tissue of the leaf and is provided with a distinct peridium of one layer of thick-walled warty cells (Fig. 1 *b*). The mean size of the æcium is 215 μ wide and 210 μ deep (range 200–270 $\times$ 180–225 μ). Aeciospores are formed in chains from a basal hymenial layer of elongated cells as in other species of *Puccinia*. They are spherical or elliptic, 17.8 μ (12–25 μ) in diameter, very lightly coloured. The spore wall is hyaline with a finely warty surface.

Telia are hypophyllous and are formed mixed with the æcia as hard-rimmed, brown, circular, open cups, 222 $\times$ 202 μ (190–250 $\times$ 180–215 μ) in diameter and depth respectively. There is no distinct peridium as in the æcium, but the cup has a lining of two to three layers of small fungal cells. Teliospores are produced from the bottom of the cup (Fig. 1 *c*) on long hyaline pedicels which get easily separated from the spores. Teliospores are two-celled subspherical, rounded at the ends, slightly constricted in the middle, deep golden brown in colour and measure 37·9 $\times$ 24·8 μ

* The first paper in this series appeared in *Proceedings of the Indian Academy of Sciences*, Section B, Vol. XXV, No. 1. In this and succeeding papers it is proposed to describe the new fungi collected from various parts of the Presidency. New records of fungi will also find a place in these papers.

(28–52× 20–28 μ). The wall is smooth and uniformly thick. Some of the spores exhibit abnormalities having three to four cells by the formation of vertical or oblique cross walls in each cell (Fig. 1 *c*).

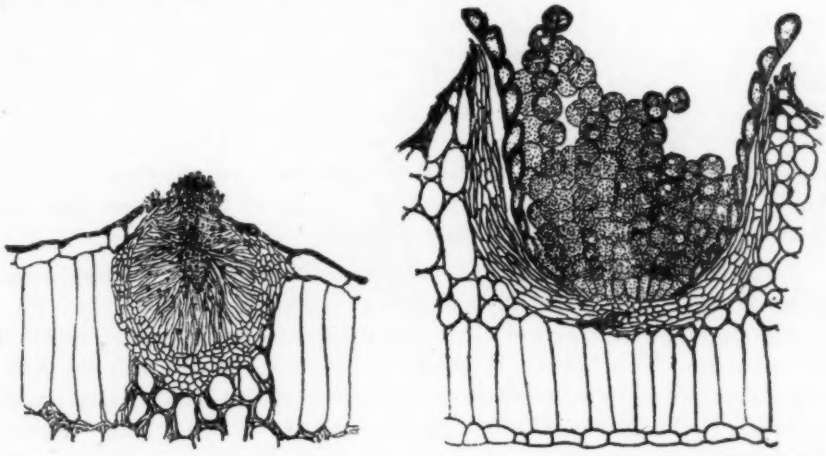
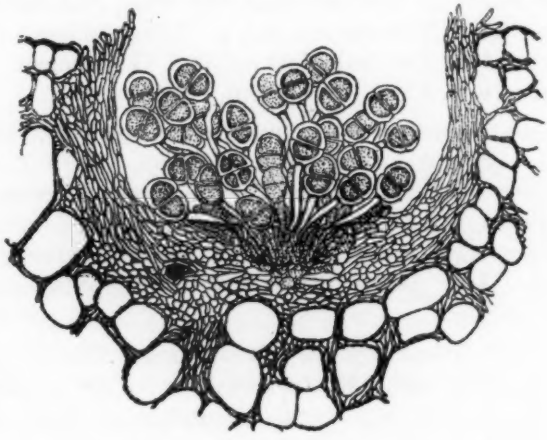


FIG. 1. (*a*) Pycnium of *P. solani-gigantea* (×360). (*b*) *Aecium* of *P. Solani-gigantea* (×360).



(*c*) Telium of *P. solani-gigantea* (× 360).

Identity of the rust.—Several rusts have been described on *Solanum*. Most of these are from the Americas or West Indies. *Puccinia solanacearum* Sacc. et Syd. has been recorded from the Sutlej valley in India. This produces 0, I, and III stages on *Solanum* sp., but unlike the rust under discussion the telia are formed only on the stem, causing malformations.

P. araucana Diet. et Neg. forms I and III and even here the telia are formed only on the stem. *P. adducta* Arth. has been recorded by Arthur (1918) on *S. racemosum* as having 0, II and III but not æcia. *P. tubulosa* Arth. (*Aecidium tubulosum* Pat et Gaill) has been observed in India on *S. melongena* but the telial stage is found to develop on *Paspalum* sp. The other species of *Puccinia* on *Solanum* have been known to form only telia. *S. giganteum* has not been known to be infected by *Puccinia* till now. The peculiar cup-like telia are also characteristic and unlike those of other species on *Solanum*. Therefore it is concluded that this rust is different and is named *P. solani-giganteæ*. Dr. G. R. Bisby to whom the specimens and the diagnosis were kindly forwarded by Dr. B. B. Mundkur, is also of the opinion that this is an unrecorded species of *Puccinia*.

Puccinia solani-giganteæ sp. nov.—*Pycnia* epiphyllous, subepidermal, globoid; *æcia* in clusters, hypophyllous, cup-shaped, 210× 215 μ with a distinct peridium of thick-walled warty cells; *æciospores* in chains, spherical or elliptical, 17·8 μ (12–25 μ); *telia* hypophyllous, mixed with the æcia, cup-shaped 220× 202 μ; *teliospores* 2-celled, deep golden brown, subspherical, smooth, 37·9× 24·8 μ (28–52× 20–28) stalked, stalks long hyaline, deciduous.

On living leaves of *Solanum giganteum* Jacq. Naduvattam (Nilgiris) 15–3–1946, Coll. C. L. Subramanian and K. Ramakrishnan. (Type) Type specimen deposited in the herbarium of the Government Mycologist, Coimbatore, and Herb. Crypt. Ind. Orient, New Delhi.

Puccinia solani-giganteæ sp. nov.—*Pycnia* epiphylla, subepidermia, globosa; *æcia* aggregata, hypophylla, cupulata, 210× 215 μ, peridio distincto cellarum crasso-muratarum; *aecio-sporidia* catenulata, globosa vel elliptica 17·8 μ (12–25 μ). *Telia* hypophylla aeciis mixta, cupulata 220× 202 μ, *teliosporidia* duo-cellata, aurati intense brunnei colores, sub-globosa, lævia, 37·9× 24·8 μ (28–52× 20–28 μ), pedicellata; pedicelli longi, hyalini, decidui.

In vivis foliis *Solani giganteæ* Naduvattam (Nilgiris), 15–III–1946, Leg. C. L. Subramanian and et K. Ramakrishnan. Typi specimina deposita in Herbario Government Mycologist Coimbatore et Herb. Crypt. Ind. Orient, New Delhi.

(5) *Entyloma bidentis* P. Henn.

Saccardo-Syll. Fung. XIV, 495, 1888.

On living leaves of *Bidens pilosa* L. Coimbatore and Kallar (Coimbatore District) October 1946 (K. Ramakrishnan).

In October 1946, this smut was noticed in an epiphytotic form on the leaves of *Bidens pilosa*, a weed, in Coimbatore and Kallar. The lower leaves were first affected and later the infection spread to the upper also. Amphigenous, whitish to yellow, circular spots develop on the leaves. The spots are small in the initial stages but enlarge later becoming 0·5 to 1 cm. in diameter. They are isolated or sometimes coalescent. The upper surface of the spot becomes convex and the lower surface correspondingly concave. The colour deepens to yellow on the upper surface and finally turns brown.

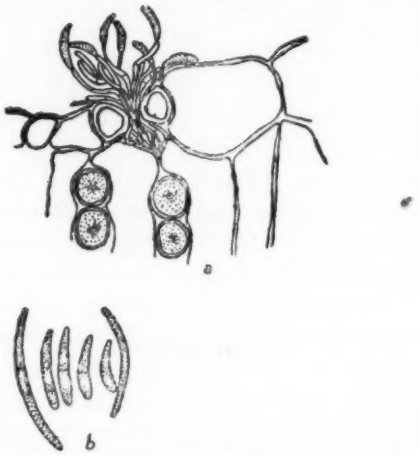


FIG. 2. (*a*) Section of leaf showing smut spores and conidia of *E. bidentis*. (*b*) conidia (× 300).

The conidia of the smut are produced when the spots have attained their full size. These are formed on both sides of the spot, but first appear on the lower surface, producing a white-dotted appearance which may deepen into greenish yellow with age. The conidia are fusiform or filiform, hyaline, straight or bent, with one to three septa. The conidiophores emerge through the stomata in fasciculate masses and bear the conidia at their apices. The conidia measure 7–8 × 1·3 μ (6–10 × 1–2 μ). The conidial fructification is like *Entylomella* V. Hohn (Ciferri, 1928).

Inoculations were carried out on healthy plants of *Bidens pilosa* with these conidia. A suspension of the conidia was made in sterilised distilled water and this was brushed over the surface of the leaves after which the plants were kept covered with a bell-jar for 48 hours. On the fifth day small white spots developed on the inoculated leaves. These enlarged and in the course of fifteen days reached their maximum diameter. The colour of the spots deepened and brown streaks became evident in twenty days. At this stage conidial formation had commenced on the lower surface of

the spots and the smut spores also had developed within the leaf. Thus the relationship between the conidia and the smut was established.

The smut spores are spherical, ellipsoidal or angular and measure on an average 15× 13 μ (11–22× 8–16 μ). The epispore is smooth but sometimes a small hyaline appendage may be seen projecting from one angle. This is only the remnant of a hypha. The spores develop intercellularly in the palisade tissue forming chains, but in the spongy tissue of the mesophyll the spores are grouped together resulting in the displacement of the cells.

Conidia have been observed in a number of species of *Entyloma*, but they have not been described in the case of *E. bidentis*.

Saccardo has listed *E. bidentis* P. Henn. as occurring on *Bidens pilosa* in East Africa. The measurements of this smut as given by Saccardo are 10–15× 9–14 μ with an epispore 1–1½ μ thick. *E. guaraniticum* Speg. has also been recorded on *Bidens pilosa* by Ciferri (1928). But the spores of this smut as described by Clinton (1904) are hyaline to light yellow with a prominent gelatinous envelope. The spores of the smut now recorded are brown and do not possess a gelatinous envelope. Owing to the difference in colour and the absence of the gelatinous envelope it is identified as *E. bidentis* P. Henn.

(6) ***Entyloma dahliæ*** Syd.

Sydow, H. and P.	..	*Ann. Mycol.* Berlin, X, 36, 1912.
Ciferri, R. de	..	*Ibid.*, XXVI, 56, 1928.
Mundkur, B. B.	..	*Trans. Brit. Mycol. Soc.*, XXIV, 332, 1940.

On leaves of *Dahlia variablis* Desf. (garden variety), Ootacamund, Nilgiris, 30–9–1946 (T. S. Ramakrishnan).

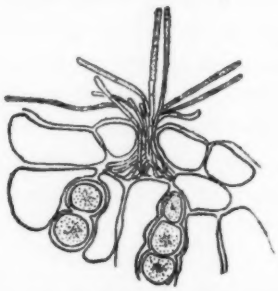


FIG. 3. Section of *Dahlia* leaf showing conidia of *E. dahliæ* (× 300).

This smut is prevalent all over the upper elevations of the Nilgiris. Mundkur has stated that conidia were not found, nor has Sydow included

them in his original description. In the specimen collected from Ootacamund conidia were observed in large numbers. These were filiform, hyaline, straight or bent. They were produced on conidiophores in fascicles emerging through the stomata on the upper surface (Fig. 3). The spots on which the conidia are formed exhibit a white ropy, reticulate appearance on the surface.

(7) *Melanotænium brachiariæ* Viegas.

Syn. *Tolyposporella brachiariæ* Mundkur and Thirumalachar, Mycological Papers, No. 16, *Imp. Myc. Inst.*, London, p. 5, 1946.

This smut has been observed in different parts of the Coimbatore District on the leaves and leaf-sheaths of *Brachiaria distachya* (L.) Stapf. (*Panicum distachyum* L.).

Viegas has described *M. brachiariæ* on the leaves of *B. plantaginea* from Brazil. Mundkur and Thirumalachar have recorded *Tolyposporella brachiariæ* from Bangalore and New Delhi. Dr. Mundkur was kind enough to let us have fragments of the type specimens of these two fungi for comparison with the specimen collected at Coimbatore. There was close agreement between the three specimens. Microtome sections of the sori (Plate XVI, Fig. *b*) revealed that the spores occur in groups in the mesophyll tissue outside the ring of cells surrounding the vascular bundles. The spore germinates producing a promycelium from the apex of which a whorl of sporidia arise (Plate XVI, Fig. *a*).

A comparison of the type specimens of *M. brachiariæ* and *T. brachiariæ* with the local specimen showed that the external symptoms, the disposition of the sori and spores and the germination of the spores were alike in all the three. The measurements of the spores were as follows:

Specimen	Range of dimensions of the spores in μ	Means size μ
M. brachiariæ ..	8–14 × 6–14	11 × 8·1
T. brachiareae ..	8–16 × 7–12	11 × 8·0
Local smut ..	8–15 × 6–11	11·5 × 9·0

It is clear from the above that the same fungus is involved in all these. It has to be decided whether it is an *Entyloma* or *Melanotænium*. The difference between these genera is very slight and rests mainly on the colour of the sori and the spores. A number of species described originally as *Entyloma* have been transferred to *Melanotænium* on this basis. Following this trend it is desirable to include this smut under *Melanotænium* on account

of the striæ-like sori and dark olive-brown spores. Dr. Mundkur's attention was drawn to the fact that the smut on *B. distachya* is not a *Tolyposporella* but a *Melanotænium* and he concurred with us in this.

(8) *Phyllachora cymbispora* sp. nov.

Spots forming yellowish green rings 1–2 mm. wide surrounding the stroma, visible on both sides of the leaf, isolated or aggregated in groups of 2–4; stromata solitary in each spot, clypeate, black, shining, and raised almost equally on both sides of the leaf, 0·3–1·5 mm., with one loculus, dense black just beneath the epidermal layers; loculus broadly-flask-shaped and flattened, ostiolate, 225–396 μ broad and 162–228 μ deep, bounded by a cellular border; *asci* thin-walled, cylindric-spindle-shaped, narrowed at the base, with a small foot, 120× 16 μ (102–150× 10–20·5); paraphyses present filiform; *ascospores* 8, light-olivaceous, cymbiform, partly distichous, 30× 6·6 μ (22–38× 3–9 μ); *pycnia* often present by the side of the perithecia, subepidermal, deeply sunk, dark-coloured, 123 μ broad and 180 μ deep, ostiolate, containing filiform, hyaline conidia (Plate XVI, Fig. *c*).

On living leaves of *Eurya japonica* Thunb., Lovedale (Nilgiris) 15–3–'46 K. Ramakrishnan and C. L. Subramanian, type. Type specimen deposited in the Herbarium of the Government Mycologist, Coimbatore and Herb. Crypt. Ind. Orient., New Delhi.

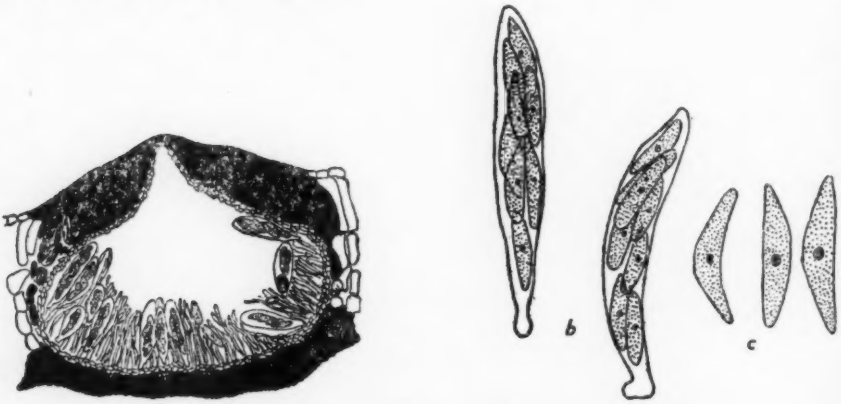


FIG. 4. (*a*) Section of perithecium of *Phyllachora cymbispora* (× 100). (*b*) Asci (× 360). (*c*) Ascospores (× 510).

Phyllachora cymbispora sp. nov. Maculæ formantes annulum flavidem viredem 1–2 mm. lat., circumdantes stroma, utrimque visibiles, solitariæ vel 2–4 aggregateæ, stroma unum in quaque macula, clypeatum, nigrum,

micans, elevatum fere pariter utrimque folii, 0·3–1·5 mm. lat., uniloculatum, ostiolatum, stroma valde nigro infra epidermium; loculus latiusculus, lageniformis, et compressus, 225–396 μ lat. 162–228 μ alt, cellis limbatus asci tenuimuniti, cylindracei fusiformes, ungustati in basi, parvo pede, 120 × 16 μ (102–150× 10–20·5) paraphyses adsunt, filiformes; ascosporidia 8, lavis olivacei colores, cymbiformes, partum disticha, 30× 6·6 μ (22–38× 3–9 μ); pycnia saepe adsunt, prope perithecia, fuscicolores 123 μ lat et 180 μ alt, ostiolata continentia filformia, conidia hyalina.

In vivis foliis *Euryæ japonicæ* Thunb., Lovedale (Nilgiris) Leg. K. Ramakrishnan et C. L. Subramanian, typus. Typi specimina deposita in Herbario Government Mycologist, Coimbatore et Herb. Crypt. Ind. Orient, New Delhi.

Catacauma euryæ (Racib) Theis. et Syd. has been recorded on *Eurya acuminata* Dc. from Java and *Phyllachora transiens* Syd. et But. on the same host from Kumaon, India. In both these fungi the stromata are hypophyllous. Further the asci and ascospores are smaller than those of *P. cymbispora*. Comparative measurements are given below.

	Asci	Ascospores
C. euryæ ..	80-90 × 14–16 μ	14–16 × 6–8 μ, hyaline, oval
P. transiens ..	50–70 × 10–11 μ	20–22 × 5–7 μ, hyaline, oblong
P. cymbispora ..	102–150 × 10–20 μ	22–38 × 3–9 μ, light olivaceous cymbiform

It is manifest that the fungus under study is quite different from those mentioned above and is therefore named *Phyllachora cymbispora* (deriving the name from the shape of the spores).

(9) *Colletotrichum ciliatum* sp. nov.

Spots amphigenous, isolated, oval, 4–9× 2–6 mm., or confluent forming irregular big patches, brownish grey with darker coloured margins; *acervuli* minute, numerous, black, amphigenous, separate, or confluent into linear striæ, erumpent; stromata of dark brown cells filling the epidermis and one or two layers of subepidermal cells, erumpent, setæ numerous, 86–135× 5–10 μ, blackish brown, pointed, 2–3 septate; *conidia* hyaline, unicellular, falcate, 29× 4·7 μ (19–25× 3–5 μ), with one terminal cilium, 5–19 μ long.

On living leaves of *Cymbopogon polyneuros* Stapf. Nanjanad (Nilgiris) 29–9–1946 (T. S. Ramakrishnan) Type.

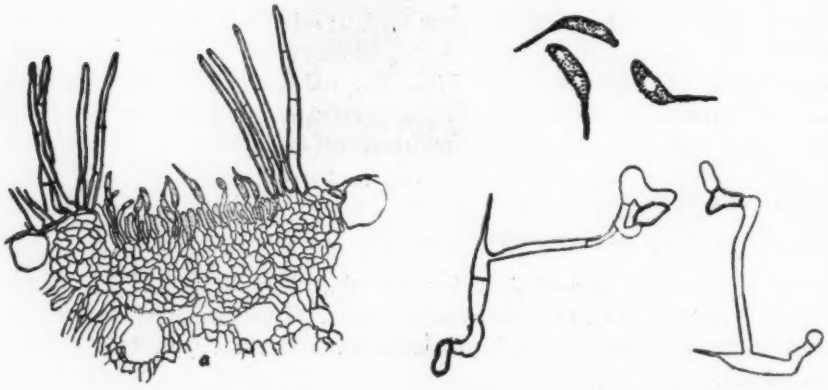


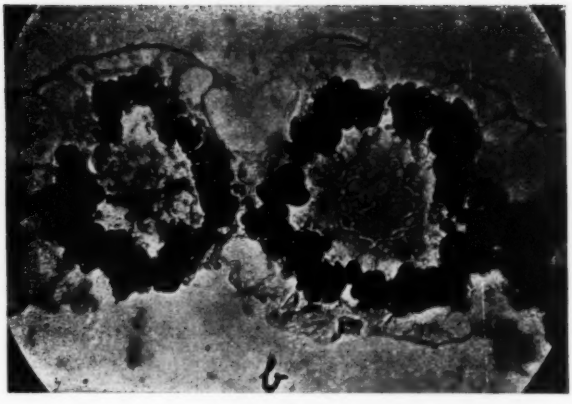
FIG. 5. (*a*) Section through an acervulus of *C. ciliatum*. (× 240). (*b*) Conidia (× 510). (*c*) Germinating conidia (× 510).

Colletotrichum ciliatum sp. nov.—Maculæ amphigeniæ, solitariæ, ovales, 4–9 × 2–6 mm., vel confluentes, formant, magna, irregularia, panna, brunneolæ, marginus fusciores; acervuli minutissimi, numerosi, fusci, amphigeni, solitariæ, vel confluentus cum liniari striis, erumpentis; stromata fuscarum brunnearum, cellarum, implentia, epidermidum et unum vel duo strata cellarum subepidermidum, erumpentia, setæ numerosæ, 86–135 × 5–10 μ, fusci brunnei colores, aculeatæ, 2–3 septatæ, conidia hyalina, unicellaria, falcata, 29 × 4·7 μ (19–25 × 3–5 μ) cum uno cilio terminali.

In vivis foliis *Cymbopogon polyneuros* Stapf. Nanjanad (Nilgiris) 29–9–1946, Leg. T. S. Ramakrishnan, typus.

This species is unique in having a cilium at the apex of the conidium. It is in the form of a sharp process and is either straight or curved. The cilium is developed as the spore matures and is not evident in the earlier stages of spore development. The germination of the conidium is as in other species of *Colletotrichum*. One or more germ tubes are produced by the conidium and appressoria develop on these. A septum is seen in some of the germinating spores. The cilium is not shed during germination nor does a germ tube develop from it. The acervuli are first seen on the upper surface of the spots. They are formed on the lower surface only in the later stages. The infected leaves soon dry up owing to the formation of large lesions. On these dry leaves acervuli may be seen all over the surface.

The characteristics of the fungus show, that it is a *Colletotrichum*. But the conidia are ciliate—a feature not noticed in the genus. However the resemblance to *Colletotrichum* is so close that it is thought fit to include



(*a*) Germinating spores of *Melanotænium brachiariæ* (×600)
(*b*) Section through the sorus of *Melanotænium brachiariæ* (×600)
(*c*) Section through the pycnidium of *Phyllachora cymbispora* (×400)

it in that genus, but as a new species. *C. graminicolum* (Ces.) Wils. has been observed on *Cymbopogon* sp., but the conidia of this species are not ciliate. Therefore the present fungus is named *C. ciliatum*.

ACKNOWLEDGMENT

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TWO SPECIES OF UNDESCRIBED LAMPYRID LARVÆ FROM S. INDIA

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I

Luciola trivandrensis sp.n. (Larva, Trivandrum, 1942)

(Figs. 1 and 2)

The following notes are based on three hitherto unrecorded larvæ.

Length.—15 mm.–20 mm.

Locality.—Trivandrum.

General colouration.—Dirty yellow.

Body is strongly depressed and dorsal plates are flat with a median whitish longitudinal sulcus.

Head is completely retractile into the thorax, about twice longer than broad, sides parallel, frontal sutures thickened into long ridges running parallel to each other up to the precoilar knobs, and metopic suture short and open. Antennæ are 3 jointed, with the basal joint very long, proximally membranous, middle joint slender, and apical joint minute with a sensory cone. Side by side with the apical joint is a sense papilla (Fig. 2, s.p.), which is remarkable in being as long as the apical joint itself. The antenna of the present larva differs from that of *L. gorhami* (i) in having the membranous proximal part of the basal joint very extensive, (ii) in the basal joint lacking the basal sclerotisation, and (iii) in the apical sensory papilla being as long as the apical joint. Mandibles are falcate and canaliculate, the molar surface bearing the characteristic hairy fringe which is not so dense as in *L. gorhami*. The retinaculum which is subacute in *L. gorhami* is entirely wanting in the present species. The labiomaxillary plate does not show any striking difference.

Pronotum is longer than wide, anteriorly narrowed and slightly notched in front. Posterior margin is almost straight, but with 2 pairs of posteriorly directed protuberances, the posterolateral pair being more rounded than the inner subdorsal pair. The inner pair of processes are continued anteriorly to a short distance as a pair of feeble carinæ. Mesonotum and

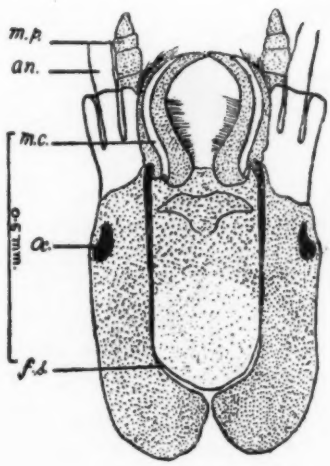

FIG. 1

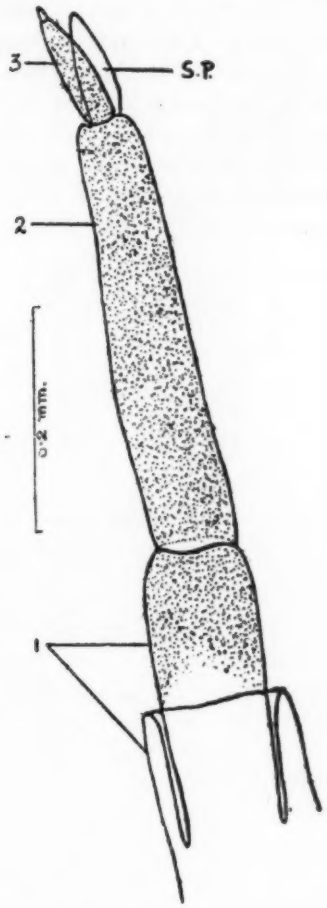

FIG. 2

FIGS. 1–2. Fig. 1. *Luciola trivandrensis* (larvæ)—head *an.* antenna, *f.s.* frontal suture. *m.c.* mandibular canal, *m.p.* maxillary palpus, *oc.* ocellus. Fig. 2. *L. trivandrensis*—antenna. 1, 2, 3, basal, middle and apical joints. *s.p.* sense papilla.

metanotum are broader than long with both pairs of processes and the subdorsal carinæ. Abdominal terga 1–7 are all broader than long and have the two pairs of posterior processes. The subdorsal carinæ form two parallel lines on the back of the larva. The eighth abdominal plate is subquadrate and without the middle pair of posterior processes, but the posterior margin is widely emarginate. The ninth plate is very narrow and always bent under the eighth.

The three larvæ collected could not be reared to the adult as they died soon. They were collected from a marshy locality in Trivandrum, S. Travancore, in April 1942. I place these larvæ provisionally under the genus *Luciola* because of their striking similarity to other described species of the genus in the occurrence of the posterior prominences, but as it is impossible to get at the most probable species out of 31 recorded Indian species of *Luciola*, I am driven to describe these larvæ provisionally as species novo, for purposes of description.

II

(?) *Pyrophanes indica* Mots.

(Figs. 3–5)

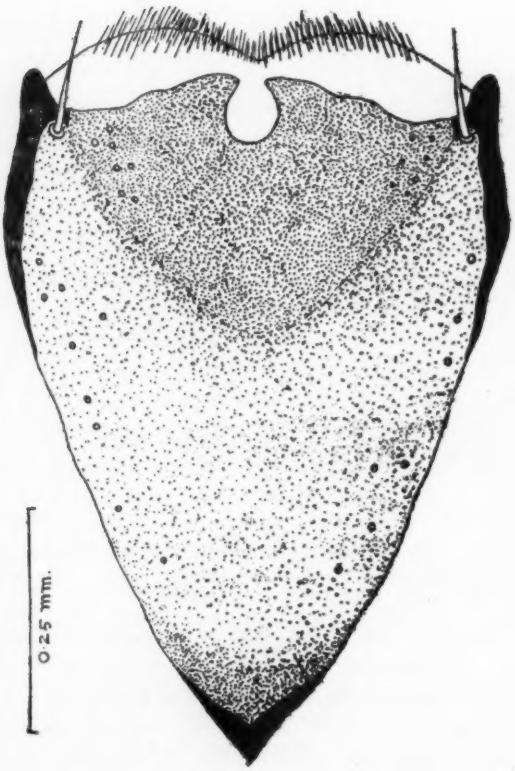

FIG. 3.

Fig. 3. (?) *Pyrophanes indica* Mots. (larvæ)—Median plate of head capsule.

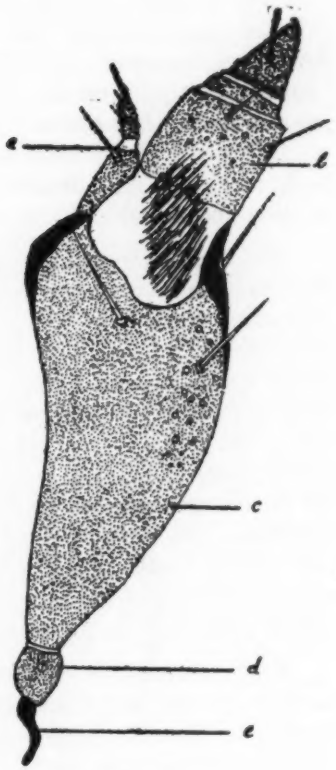

FIG. 4. FIG. 5.

Fig. 4. *P. indica*—antenna (*a*) ocellus, (*b*) head capsule. Fig. 5. *P. indica*—Maxilla (ventral view): (*a*) galea, (*b*) palpus, (*c*) stipes, (*d*) alacardo, (*e*) subcardo.

Length.—6 mm.–17 mm.

Locality.—Tambaram.

Colour.—Black with castaneous patches.

Head is chestnut brown with blackish shades. Epicranial suture is open throughout, the frontal sutures running up to free margin of the head capsule, which is thus divided into a median frontoclypeus and lateral parietals. Metopic suture is constricted at the middle. Frontoclypeus is roughly triangular with the base placed in front of the parietals. It is deeply sinuate anteromedially and thickened laterally forming an anterolateral precoila

for mandibular articulation. Anteriorly the parietal gives off on the ventral side the mandibular apophyses which carry the acetabula for articulation with the condylar postartis of mandible. The hypostomal ridges possess a broad thickening near the middle. Ocelli are borne on open sockets. Antenna is 3-jointed, basal joint with a distal chitinous ring, the middle joint completely chitinised and terminal joint minute with 4 or 5 setæ. This larva resembles *L. gorhami* Rits. in its 3-jointed antenna, but differs from it in the absence of the basal sclerotisation and subglobular sensory papilla. In *L. tenebrosus* Wlk. antenna is 2-jointed, the basal with very feeble chitinisation and apical with two sensory papillæ.

The buccopharyngeal apparatus includes the labrum, hypopharynx and pharynx. Labrum is semimembranous with its free margin dorsally visible and folded beneath to be attached dorsal to the base of hypopharynx. It consists of two pilose lobes separated by a notch and is strengthened by a minute central sclerite. Hypopharynx is a thin transverse membranous lobe lying in front of the pharynx close to the base of the mandibles. It is trilobed, the median lobe being suppressed and lateral lobes setose. The hypopharyngeal bracons are a pair of transverse chitinous bars united in the middle and attached to the hypostomal ridge close to the mandibular apophyses. Pharynx as usual presents two parts. Mandibles are falcate, canaliculate, basally dilated and setose and with a long seta just behind the external opening of the mandibular canal. It differs from *L. gorhami* Rits and *Diaphanes* sp. in the absence of a sharp retinaculum.

At the base of the labiomaxillary plate is the transverse gular plate. Cardo is divided into a proximal narrow curved thick *subcardo* and a distal broad thin *alacardo* which is attached to the massive stipes. Stipes bears three long ventral setæ, two near the anterior border and one near the middle. Maxillary palpus tapers distally and is 4-jointed, basal stout with few long setæ, second transverse, third very narrow, and fourth conical. In *L. tenebrosus* Wlk. the maxillary palpus is 3-jointed. The galea is 2-jointed, basal long and cylindrical with one or more long setæ and distal with one long apical seta besides a few short setæ. Lacinia is in the form of a dorsal densely setose cutting edge. Postlabial sclerite is long and deeply forked behind and carries two long symmetrically placed setæ. Each lobe of the bifid prelabium is provided ventrally with a pair of setæ, outer one being longer than the inner. Dorsally there is a pair of brush-like hairy growths which represent the paraglossæ. Labial palpus is 2-jointed.

Thorax.—The ventral thoracic sclerites are ill-defined. On either side of the rudimentary prosternum is a slender obliquely placed cervical sclerite

which gives off two long processes. Trochanter is undivided. Tergites are deeply arched, without a mid-dorsal sulcus and very slightly cleft anteromedially. Pronotum carries two castaneous fasciæ. Mesonotum and metanotum are black with a castaneous central area.

Abdomen.—Dorsally there are nine distinct tergites, each with a pair of lateral lobes and anterolateral notches, which are wanting or ill-defined in the first and last tergites. All the tergites possess a median castaneous area. The lateral lobes also carry a similarly coloured area which in the first tergite is not clearly visible and in tergites 7, 8 and 9, it occupies almost the whole surface of the lobe. Ninth tergite carries posteriorly a single marginal row of from 10–16 stiff setæ. Abdominal tergites 2–8 have each a pair of conspicuous perforations,* the nature and functions of which are obscure. Each pleurite is elongate and forked behind, the dorsal or outer process of which being shorter than the setose ventral or inner process. The spiracle lies in the fork. In some larger specimens a second row of very minute pleurites are observed to lie between the sternites and spiracular pleurites. Sternites 2–6 are widely emarginate laterally and anteriorly, the anterolateral and posterolateral angles being produced. Sternites 7–9 are paler and less emarginate laterally. Ninth sternite carries four groups of setæ. Lying posterior to the ninth is an incomplete chitinous ring supporting the anal brush. Photogenic organs are paired and placed on the seventh abdominal segment. The pale green light is seen dorsally also through the pale lobes of the corresponding tergite. The anal brush consists of a circlet of creamy white retractile filaments, arranged in four bundles with four primary filaments in each bundle. Each filament is bifurcate and clothed with several minute recurved hooks.

Internal Anatomy.—The narrow œsophagus opens into the gizzard which leads into a long convoluted mid-gut. The four Malpighian tubules form a coiled mass. A pair of connectives run from the brain to the sub-œsophageal ganglion which is more or less triangular. It is followed by three globular and equidistantly placed thoracic ganglia. Abdominal ganglia are eight. The metathoracic and first abdominal ganglia are not very much approximated. The last two ganglia are slightly approximated.

The special features of interest in the anatomy of the present larva are the open ocular fossa, divided cardo, complete division of the head-capsule, absence of mid-dorsal sulcus, deeply arched tergites, presence of marginal row of setæ in ninth abdominal tergite and the presence of lateral perforations in abdominal tergites 2–8.

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* These perforations are noticed in the living larva as minute creamy white specks.

This species is fairly abundant in certain months of the year especially in September and October. The larvæ were first collected by me in September 1941 from Tambaram. They were reared several times within the laboratory but most of them moulted once and then died. They were first mistaken for the very early stages of *L. tenebrosus* Wlk. which are also available in the same locality. A detailed study of its morphology has revealed it to be entirely different from all the other lampyrid larvæ described so far. Most probably it is related to *Luciola* and belongs to the sub-family Luciolini. The only other Indian lampyrid genus of this sub-family is *Pyrophanes*, " the early stages of which appear to be quite unknown " (Blair, 1927). The only Indian species of this genus described is *P. indica* Mots.

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